CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-813

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 21-813	Submission Date(s): 02/16/2006
Brand Name	Bio-E-Gel®
Generic Name	Estradiol gel 0.06%
Reviewer	Doanh Tran, Ph.D.
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OCP Division	Division of Clinical Pharmacology 3
ORM division	Division of Reproductive and Urology Products
Sponsor	BioSante Pharmaceuticals, Inc.
Relevant IND(s)	IND 51-229
Submission Type; Code	Original NDA (3S)
Formulation; Strength(s)	Topical gel, 0.06% w/w
Indication	Treatment of moderate-to-severe VMS [ ]

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1 Executive Summary

1.1 Recommendation

This reviewer finds NDA 21-813 acceptable from a clinical pharmacology perspective provided the labeling comments are adequately addressed.

1.2 Phase IV Commitments

None

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Bio-E-Gel is a transdermal gel formulation of estradiol (0.06% w/w) intended for the treatment of moderate-to-severe vasomotor symptoms (VMS) [ ] associated with menopause. The NDA contains 4 supporting phase 1 studies examining the pharmacokinetics of Bio-E-Gel (EST003, EST006, EST007, and EST008. Additional studies [ ] used a prototype formulation and were not reviewed), 1 phase 2 dose finding study (EST004), and 1 phase 3 efficacy and safety study (EST005). Estradiol exposure at all proposed doses was available mainly from phase 1 studies EST007 and EST008 and pre-dose samplings at weeks 4, 8, and 12 in the phase 3 trial EST005.

The proposed doses of Bio-E-Gel are 0.87, 1.7 [ ] g/day, containing 0.52, 1.02 [ ] mg/dose estradiol, and delivering systemically [ ] mg/24 hours estradiol. Estradiol exposure linearly increased with dose but at a higher than proportional rate. Exposure to the proposed doses of Bio-E-Gel is within the range of currently approved topical products. Furthermore, the 0.87 g/day dose would deliver a rate lower than any other approved product.

Estradiol 24-hour concentration profile is variable with apparent plateaus at steady state for the lower 2 dose levels. The profile for the 2.6 g/day was limited by reduced number of sampling points. Apparent elimination half-life is prolonged (means of 55 – 75 hours) and is likely due to slow absorption rate and endogenous production of estradiol. Distribution, metabolism, and excretion of estradiol were not examined in this NDA but are well known.

Estradiol metabolites estrone and estrone sulfate concentrations also increased with Bio-E-Gel application. However, the baseline-unadjusted estradiol:estrone ratio also increased with dose. The estradiol:estrone ratios were 0.53, 0.98, and 1.3 for 0.87, 1.7, and 2.6 g/day doses, respectively.

Application of sunscreen 10 minutes before application of Bio-E-Gel increased the exposure to estradiol by approximately 55%. No significant change in estradiol exposure was observed when sunscreen was applied 25 minutes after application of Bio-E-Gel. In the same study, prolonged (7 days) concomitant application of sunscreen to the site of Bio-E-Gel application increased exposure to estradiol by about 2-fold, regardless of whether it was applied before or after application of Bio-E-Gel. About 15% of the increase may be attributed to the increase in SHBG concentration. The cause of the remaining increase in not known.

Exogenous administration of estrogens, particularly via the oral route could significantly increase the concentration of serum hormone binding globulin (SHBG). The lower 2 doses of Bio-E-Gel only slightly and not statistically significantly increased mean SHBG concentration by less than 15% following 12 weeks of exposure. The 2.6 g/day dose significantly increased SHBG concentration to a maximum mean increase of 37% after 8 weeks.

Examination of the responses due to Bio-E-Gel suggested that the reduction in frequency and severity of VMS was dose dependent. [ ]
In term of safety, dose dependent rate of common adverse effects in the reproductive and breast class was observed as would be anticipated. There was also a high rate of the significant adverse effect endometrial hyperplasia (11.1%) in the 2.6 g/day dose group. [ ]
[ ]

There was no observed change in estradiol concentration in male partner following direct contact at 2 and 8 hours post Bio-E-Gel application. Less than 10% of applied estradiol was recovered on application site at 2 and 8 hours post application. Washing the area with soap and water reduced residual skin estradiol to about /% of applied dose.

The formulations used in all supporting trials are identical to the to-be-marketed formulation.

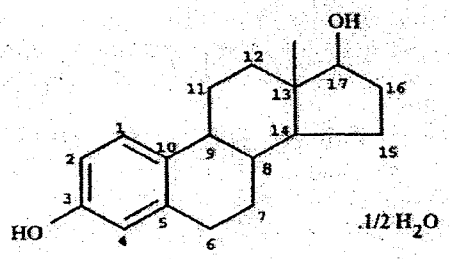
2 Question Based Review

2.1 General Attributes

2.1.1 What is Bio-E-Gel?

Bio-E-Gel is a topical formulation of estradiol, an estrogen sex hormone that is used to treat menopausal symptoms in women. Bio-E-Gel is a homogeneous, transparent and non-staining hydroalcoholic gel, containing estradiol 0.06% w/w. (Note: this review refers to the drug product in this NDA as Bio-E-Gel. [ ].)

The structural formula of estradiol is:



Estrogens are a group of hormones that play an important role in normal sexual and reproductive development in women. Of the 3 active estrogens (estradiol, estrone, and estriol), estradiol is most potent. The estrogenic potency of estradiol is 12 times that of estrone and 80 times that of estriol. Estradiol is primarily converted to estrone by 17-beta-hydroxysteroid dehydrogenase. Estrone undergoes conversion by 16-alpha-hydroxylation and 17-keto reduction to estriol, which is the major urinary metabolite. A variety of sulfate and glucuronide conjugates also are excreted in the urine (In: Goodman and Gilman's, 9th edition).

In premenopausal women, during the early follicular stages both estradiol and its metabolite estrone serum concentration are typically between 40 and 60 pg/mL, with estradiol increasing to 200 to 400 pg/mL and estrone to 170 to 200 pg/mL during the late follicular phase. After menopause, serum estradiol level is reduced to about 5 to 20 pg/mL and estrone concentrations between 30 and 70 pg/mL.

2.1.2 What are the proposed indications of Bio-E-Gel?

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

[ ]

2.1.3 What are vasomotor symptoms (VMS) and vulvar and vaginal atrophy (VVA)?

Vasomotor symptoms comprise the symptoms commonly known as 'hot flush'. It is the hallmark of menopause. Hot flushes occur in 75 – 85% of postmenopausal women for an average duration of 1 – 2 years. The cause of VMS is unknown but believed to occur due to induced lability in the thermoregulatory center of the hypothalamus with declining levels of estrogen and progesterone resulting in peripheral vasodilation. Characteristics of hot flush includes sudden onset of reddening of the skin over the head, neck, and chest. A feeling of intense body heat with duration of a few seconds to minutes, and rarely for up to an hour, that concludes by sometimes profuse perspiration. The frequency may be rare to recurrent every few minutes. Each episode coincides with a surge in LH. The severity is defined as follow:

Mild: sensation of heat without sweating

				12
EST006	2.6, 5.2	Single dose, transfer study	Upper arm	PK in untreated male partner only
EST007	0.87, 1.7	14 days	Upper arm	Day 1, Day 14, and trough levels in between
EST008	2.6	15 days	Upper arm	Day 15 (before sunscreen period begins)

Table 2: Formulations used in the development of Bio-E-Gel. The proposed commercial formulation is the same as the formulation used in the phase 3 efficacy trial.

Component	Function	Prototype Formulation (% w/w)	Proposed Commercial Formulation (% w/w)
Estradiol	Active	0.06%	0.06%
Ethanol, [ ]		[ ]	[ ]
Propylene Glycol			
Diethylene glycol monoethyl ether [ ]			
Carbomer 940 [ ]			
Triethanolamine [ ]			
Purified Water			
Edetate Disodium [ ]			

2.2 General Clinical Pharmacology

2.2.1 What are the pharmacokinetic (PK) characteristics of the drug and its major metabolites?

PK of estradiol (E2), estrone (E1), and estrone sulfate (E1-S) from Bio-E-Gel were characterized mainly in 3 studies, namely EST003, EST007, and EST008. EST003 examined the PK following once daily doses of 1.25 and 2.5 g Bio-E-Gel for 14 days applied to the front and inner thigh (Data not shown since site of application and dose are different than the proposed site and dose, see appendix for study review). EST007 examined once daily doses of 0.87 and 1.7 g Bio-E-Gel for 14 days applied to the upper arm. Both studies examined PK profiles following the first dose and 14th dose as well as trough levels of most days in between. Additionally, PK of the 2.6 g dose may be obtained from the no-sunscreen arm in study EST008 where Bio-E-Gel 2.6 g was applied to the upper arm once daily for 15 days.

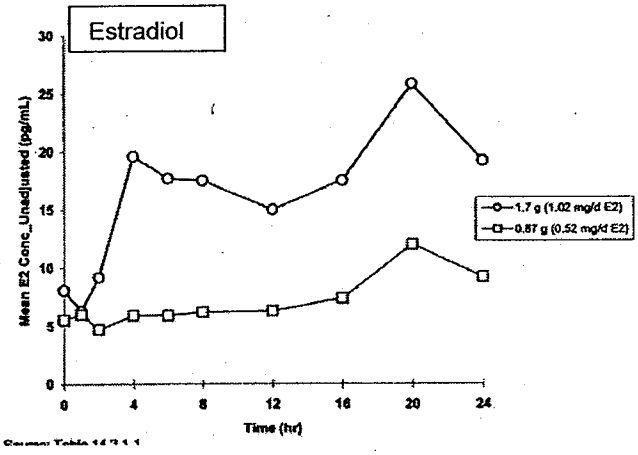
PK profiles for 0.87 and 1.7 g/day doses:

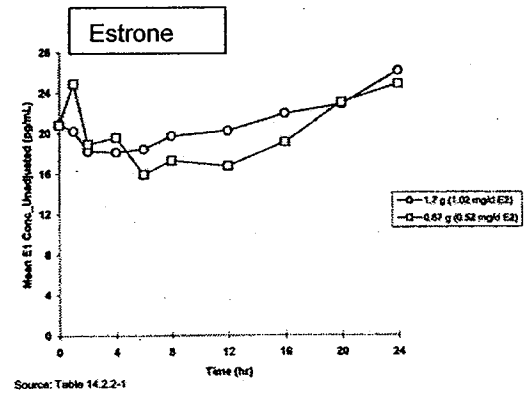
The following 3 figures shows the mean baseline-unadjusted serum concentrations for E2, E1, E1-S following 0.87 and 1.7 g/day Bio-E-Gel administration. Figure 1 shows 24-hour concentration profiles following the first dose of Bio-E-Gel. Estradiol concentration is variable but increased following application of Bio-E-Gel. There appears to be a plateau of concentration that did not decline to a clear trough before the next dose. The sharp changes in concentrations from

20 to 24 hour time points are likely the results of data variability. Figure 2 shows the pre-dose concentrations measured on most days in the 14 daily dose study. Median pre-dose concentrations suggest that steady state concentration of E2 was reached after 3 days. The mean pre-dose concentrations appear relatively stable after Day 4, consistent with being at steady state. Figure 3 shows 24-hour concentration profiles at steady state (Day 14). Mean estradiol levels were higher in the 1.7 g dose compared to the 0.87 g dose. Figures 1 – 3 also include estrone and estrone sulfate profiles, which generally paralleled that of estradiol. Data are presented as total non-baseline-adjusted concentrations unless specified otherwise.

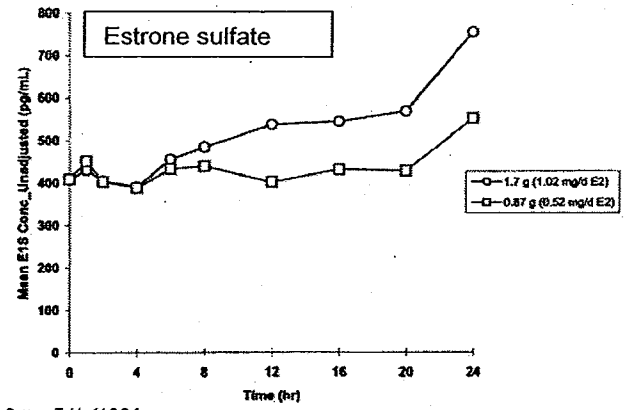
The mean steady state (Day 14) data (fig. 3) is reflective of the variability in individual PK profiles. The features observed in the mean data profile can be seen in many of the individual profiles. For example, 6 out of 10 profiles from the 1.7 g dose group appear to have small elevated bump in E2 concentration at 4 – 6 hour and 5 out of 8 profiles had a bump at 20 hour (2 individuals had outlier at 16 or 20-hour collection and was not used for this analysis). The intraday variability of mean E2 concentrations on Day 14 is greater in the 1.7 g dose group as compared to the 0.87 g group. There also was a greater inter subject variability in the 1.7 g dose group relative to the 0.87 g dose group with mean CV% of 69% and 52%, respectively (see figure 5 for standard deviations). For both dose levels, there were no clear plateaus in the individual trough concentrations over 14 days. This may be expected since intraday PK profiles suggest that the samplings just prior to next dose, which is being used as trough concentrations, do not have lowest concentrations.

Figure 1: Mean unadjusted serum E2, E1, and E1-S concentrations after a single dose of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel.



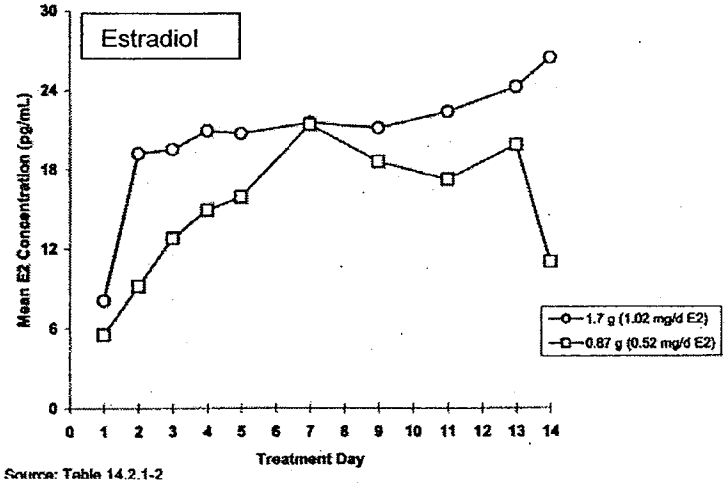


Source: Table 14.2.2-1



Source: Table 14.2.3-1

Figure 2: Mean unadjusted trough serum E2, E1, and E1-S concentrations of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 1-14).



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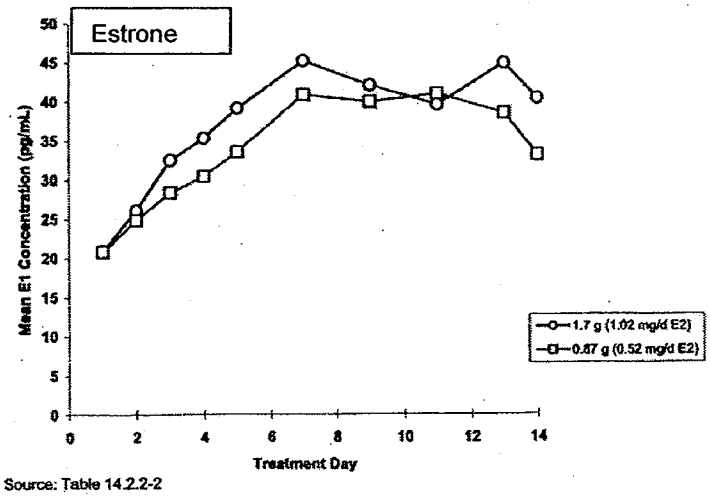

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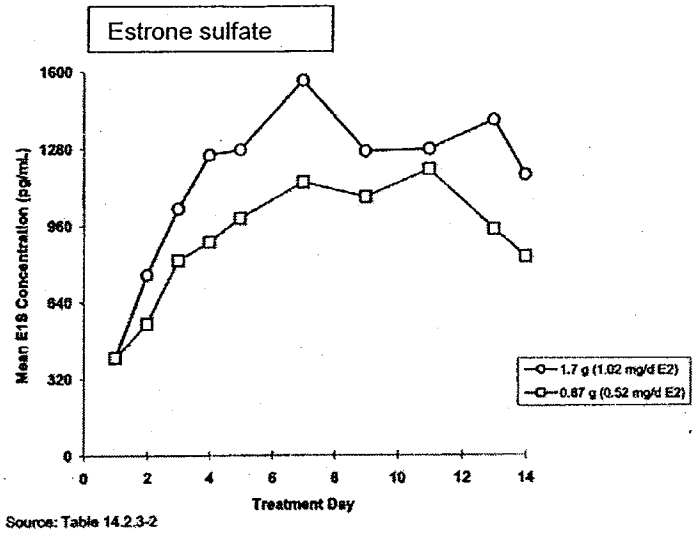
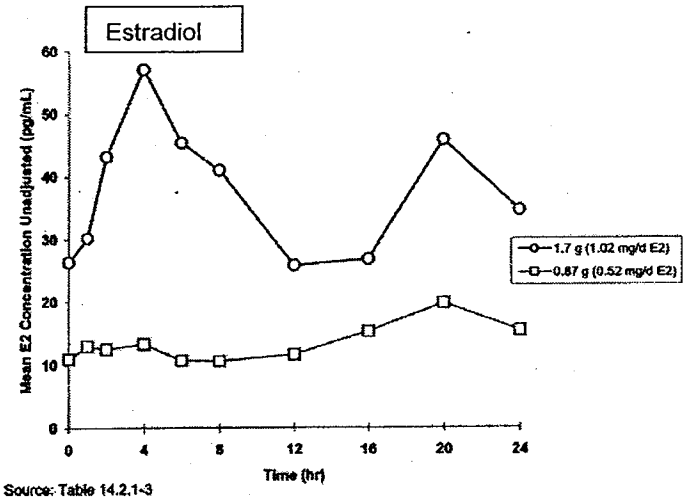


Source: Table 14.2.3-2

Figure 3: Mean unadjusted serum E2, E1, and E1-S concentrations after multiple doses of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 14).



Source: Table 14.2.1-3

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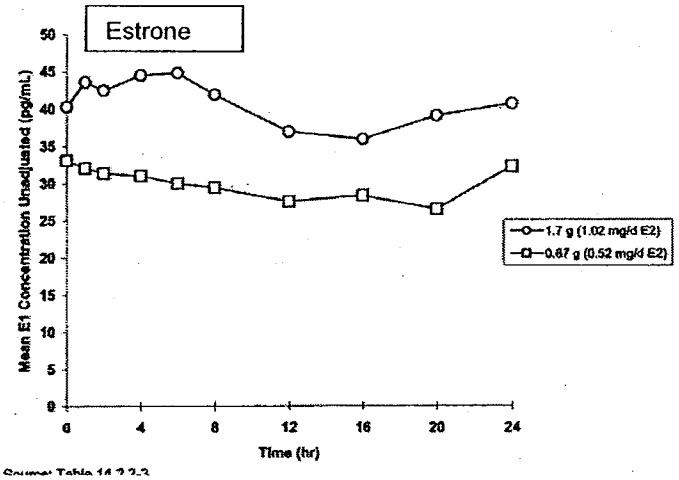
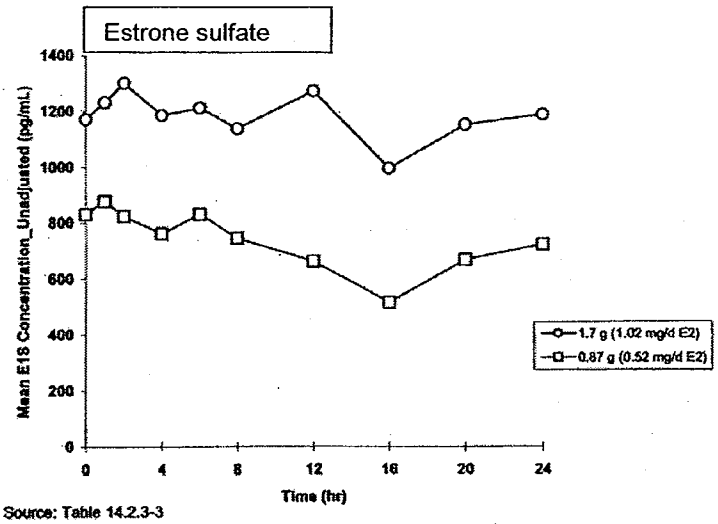




Source: Table 14.2.3-3

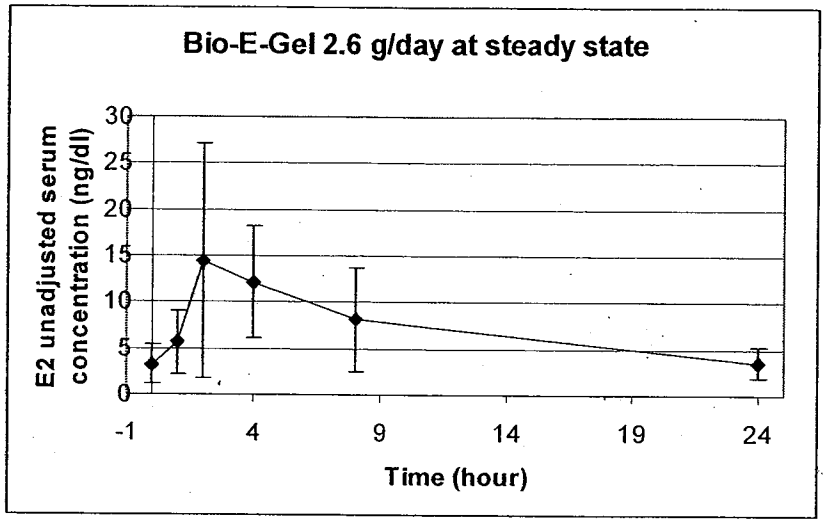
PK profiles for 2.6 g/day dose:

For the 2.6 g/day dose, the sponsor did not perform a separate PK study as above to determine its PK. However, the steady state PK for the 2.6 g/day dose could be obtained from the Bio-E-Gel only control groups in the sunscreen effect study (EST008). Steady state was assumed to be reached by the PK measurement day in this study (i.e., Day 15) based on the time to steady state of about 3 days observed previously for the lower doses. This study contained 2 groups of 6 subjects that were dosed with 2.6 g/day Bio-E-Gel to the upper arm for 15 consecutive days. Data from these subjects were pooled and plotted in the figure below. This study has a less intensive sampling regimen and may miss any peak that occurs between 12 and 20 hour post dosing. Figure 4 below shows the PK profiles following 2.6 g/day Bio-E-Gel at steady state (Day 15).

Bio-E-Gel 2.6 g/day dose yielded greater E2 concentrations than both lower doses of 0.87 and 1.7 g/day (fig. 5). The 0h and 24h timepoints had similar mean concentrations of about 32 and 36 pg/ml, respectively, indicating steady state predose level greater than endogenous baseline E2

and a return to steady state "trough" at 24h. There was a substantial (4.4-fold) mean increase from 0-h to 2-h that was observed at a less extreme extent in the 1.7 g/day dose and not observed at the lowest dose. This data suggests a relationship of dose and time to first peak as well as extent of peak concentration increase. As a result, higher dose of Bio-E-Gel exhibits a wider range of steady state E2 concentrations.

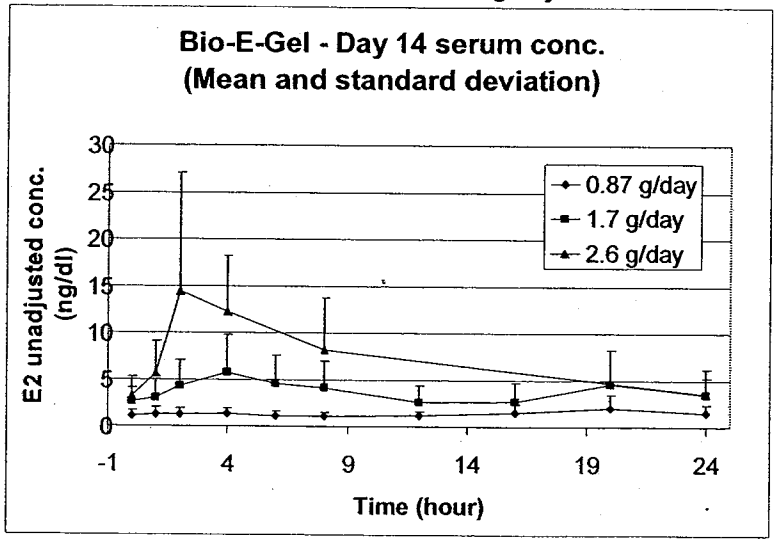
Figure 4: Mean (data points) and standard deviation (error bars) of unadjusted E2 serum concentration following 15 consecutive daily dose of 2.6 g/day Bio-E-Gel.



Note: PK profiles for estrone and estrone sulfate for the 2.6 g/day dose are also available in the NDA. They generally parallel that of E2 and are not included here to be brief.

The Estradiol steady state concentration for all [redacted] doses are shown in the figure below.

Figure 5: Unadjusted E2 concentration at steady state (Day 14 or 15) following daily dosing of Bio-E-Gel at doses of 0.87, 1.7 and 2.6 g/day.



Apparent half-life.

The apparent half-life ($t_{½}$) of estradiol adjusted for baseline endogenous serum estradiol concentrations was reported for the doses studied in EST007 and EST003. When adjusted for baseline endogenous serum estradiol concentrations, the apparent $t_{½}$ after the last daily application of Bio-E-Gel was 54.8 ± 40.3 h for the 0.87 g/day dose and 75.2 ± 146.7 h for the 1.7 g/day dose (EST007). These half-life values are much longer than the known short biological $t_{½}$ of estradiol (e.g., minutes (In: Goodman and Gilman's, 9th edition)), which was suggested by sponsor to likely reflect the confounding factors of prolonged absorption of estradiol from gel through the skin and by endogenous estradiol production.

In comparison, following application to the thigh, the apparent $t_{½}$ of estradiol adjusted for baseline endogenous serum estradiol concentrations were lower at 40.3 ± 31.6 h for 1.25 g/day dose and 37.3 ± 12.4 h for 2.5 g/day dose (EST003). The different $t_{½}$ between studies EST007 and EST003 suggests that application sites (arm vs. thigh) may potentially have an effect on the absorption of Bio-E-Gel.

Effect of Bio-E-Gel administration on SHBG concentrations.

Modest increase in SHBG concentrations were observed for doses of 1.7 g/day and lower. A higher dose of 2.5 g/day showed a mean increase of 21% after 15 daily applications. The highest dose of 2.6 g/day also resulted in the highest increase of 37% at week 8 in a 12-week study.

SHBG levels were determined after 14 once daily applications of 1.25 and 2.5 g/day Bio-E-Gel in study EST003. SHBG concentrations were measured at baseline (Days -16 and -10), pre-dose on Day 9, and Day 16, 0 hour. 1.25 g/day Bio-E-Gel only increased SHBG concentration by about 10% on Day 9 and about 16 % on Day 16. 2.5 g/day Bio-E-Gel increased SHBG concentration by about 6% on Day 9 and about 21% on Day 16 (Table 3).

Table 3: SHBG concentration (nM) (EST003)

Treatment	Statistic	Scheduled time			
		-16	-10	192 (Day 9 predose)	360 (Day 16, 0 H)
Bio-E-Gel, 1.25 g (0.75 mg E2)	N	6	6	6	6
	Mean	72.33	72.50	80.17	84.00
	SD	23.73	24.83	28.53	29.18
	GeoM	69.12	69.02	75.94	79.73
	G_CV	34.09	35.54	37.59	36.85
Bio-E-Gel, 2.5 g (1.5 mg E2)	N	6	6	6	6
	Mean	74.00	72.50	77.83	88.83
	SD	29.64	30.34	32.17	38.97
	GeoM	68.91	67.20	72.20	81.17
	G_CV	43.88	45.10	45.01	50.71

In the phase 3 trial, pre-dose SHBG levels were determined on Day 1 (baseline) and after 4, 8, and 12 weeks of daily Bio-E-Gel administration. SHBG increased slightly (<15%) with 0.87 and 1.7 g/day dose and the greatest increase was seen with the 2.6 g/day dose, where SHBG reached a maximum and statistically significant increase of 37% at week 8 (Table 4 and fig. 6).

Table 4: Trough serum concentrations of SHBG over time (EST005).

Hormone [Normal Range][a]	Evaluation	Placebo	Bio-E-Gel 0.87 g/day	Bio-E-Gel 1.7 g/day	Bio-E-Gel 2.6 g/day
SHBG [40-120] (nmol/L	Day 1, N	136	135	142	68
	Mean ± SD	88.0 ± 43.2	88.8 ± 48.8	87.8 ± 38.8	80.7 ± 35.5
	Median	81.0	76.0	80.5	80.0
	Range	[			]
	Day 29 (Week 4), N	132	131	137	68
	Mean ± SD	85.7 ± 42.6	92.7 ± 48.3	94.2 ± 41.0	104.9 ± 48.6†
	Median	77.0	85.0	85.0	100.5
	Range	[			]
	Day 57 (Week 8), N	125	131	134	64
	Mean ± SD	85.4 ± 37.9	93.5 ± 50.6	99.0 ± 42.9	111.4 ± 52.9*
	Median	80.0	84.0	90.5	111.5
	Range	[			]
	Day 85 (Week 12), N	132	132	138	67
	Mean ± SD	87.2 ± 44.1	96.4 ± 52.7	99.1 ± 42.2	111.0 ± 51.0*
	Median	77.5	89.0	95.5	107.0
	Range	[			]

[a] Normal range is for premenopausal women (see Section 9.5.4)

†$P<0.05$, *$P<0.01$, **$P<0.001$, ***$P<0.0001$ for comparison of LS means for each Bio-E-Gel treatment group with placebo (Dunnett's test).

Cross Reference: Study ESST005 CSR Stat. Table 14.2.54, Appendix 16.2.5.4

Trough E2, E1, and E1-S levels in phase 3 trial patients.

Trough E2, E1, E1-S, and SHBG levels were collected in the phase 3 trial (EST005) at baseline, 4 weeks, 8 weeks, and 12 weeks after start of Bio-E-Gel therapy. The figure below shows the median values at baseline (Day 1), week 4, week 8, and week 12 for patients receiving placebo, or 0.87, 1.7, and 2.6 g/day Bio-E-Gel. E2, E1, and E1-S levels were similar from week 4 to 12. Higher dose of Bio-E-Gel resulted in higher concentrations of E2, E1, and E1-S. The median E2, E1, and E1-S were similar between week 4 and week twelve, suggesting that steady state was reached by week 4 in these patients. Note that the individual levels were highly variable with a large range (see Table 5). Also the mean estradiol steady state pre-dose concentrations did not always increase with increasing Bio-E-Gel dose. However, they are always clearly higher than placebo.

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Figure 6: Median Trough Serum Concentrations of Estradiol, Estrone, Estrone Sulfate, and SHBG (Study EST005) (ITT -Observed Data Set).

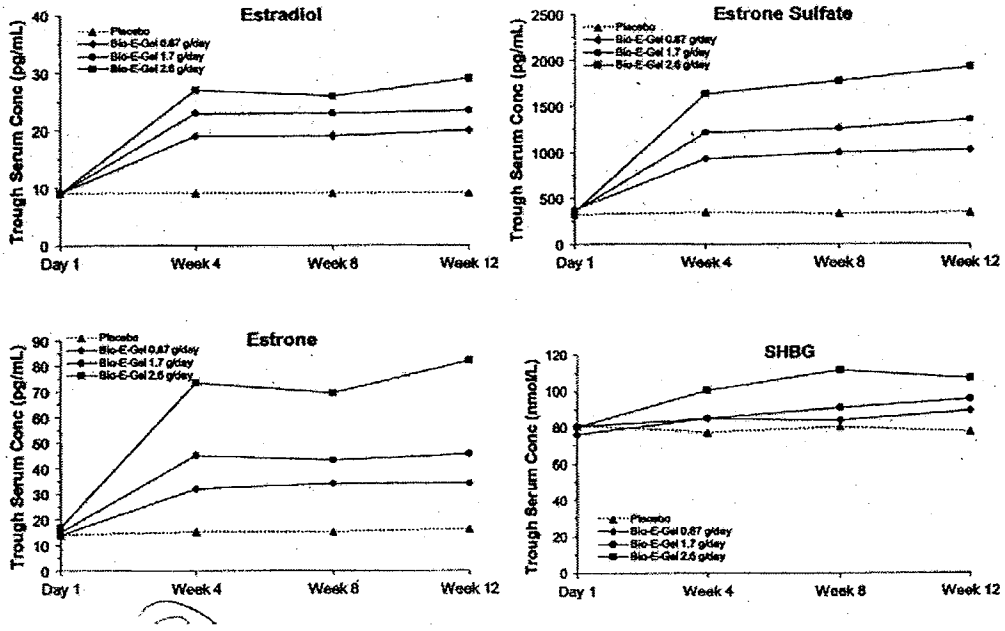

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Table 5: Trough serum concentrations of estradiol, estrone, and estrone sulfate over time (EST005).

Hormone [Normal Range][a]	Evaluation	Placebo	Bio-E-Gel 0.87 g/day	Bio-E-Gel 1.7 g/day	Bio-E-Gel 2.6 g/day
Estradiol [30-300] (pg/mL)	Day 1, N	136	133	142	68
	Mean ± SD	12.4 ± 10.6	13.2 ± 14.8	11.2 ± 10.1	9.7 ± 2.9
	Median	9.0	9.0	9.0	9.0
	Range				
	Day 29 (Week 4), N	131	130	136	67
	Mean ± SD	13.3 ± 18.1	30.4 ± 35.3†	41.7 ± 73.4***	35.8 ± 33.8*
	Median	9.0	19.0	23.0	27.0
	Range				
	Day 57 (Week 8), N	123	130	132	64
	Mean ± SD	12.3 ± 15.6	31.0 ± 35.5†	42.7 ± 99.0**	41.1 ± 41.9*
	Median	9.0	19.0	23.0	26.0
	Range				
	Day 85 (Week 12), N	128	131	138	67
	Mean ± SD	14.3 ± 28.0	34.3 ± 43.8**	38.7 ± 42.3***	40.8 ± 37.1***
	Median	9.0	20.0	23.5	29.0
	Range				
Estrone [30-160] (pg/mL	Day 1, N	135	130	139	67
	Mean ± SD	16.1 ± 10.4	15.5 ± 7.9	16.2 ± 8.5	19.2 ± 11.0
	Median	14.0	14.0	15.0	17.0
	Range				
	Day 29 (Week 4), N	130	125	133	64
	Mean ± SD	17.8 ± 24.9	35.4 ± 20.3***	47.1 ± 26.9***	78.0 ± 44.6***
	Median	15.0	32.0	45.0	73.5
	Range				
	Day 57 (Week 8), N	123	127	129	62
	Mean ± SD	17.2 ± 18.9	37.6 ± 20.4***	49.9 ± 28.8***	82.6 ± 48.7***
	Median	15.0	34.0	43.0	69.5
	Range				
	Day 85 (Week 12), N	127	126	132	66
	Mean ± SD	19.4 ± 17.6	39.7 ± 24.2***	51.6 ± 30.9***	85.5 ± 52.6***
	Median	16.0	34.0	45.5	82.0
	Range				
Estrone sulfate [650-3600] (pg/mL	Day 1, N	136	134	142	68
	Mean ± SD	401 ± 273	438 ± 257	370 ± 161	450 ± 471
	Median	320	370	355	350
	Range				
	Day 29 (Week 4), N	131	132	136	68
	Mean ± SD	421 ± 457	1185 ± 1083***	1359 ± 804***	2213 ± 1844***
	Median	340	930	1210	1635
	Range				
	Day 57 (Week 8), N	125	129	133	64
	Mean ± SD	400 ± 333	1203 ± 878***	1446 ± 964***	2201 ± 1735***
	Median	330	1000	1260	1780
	Range				
	Day 85 (Week 12), N	130	128	136	67
	Mean ± SD	444± 424	1338 ± 1277***	1464 ± 1028***	2284 ± 1792***
	Median	345	1030	1355	1930
	Range				

[a] Normal range is for premenopausal women (see Section 9.5.4)

†$P<0.05$, *$P<0.01$, **$P<0.001$, ***$P<0.0001$ for comparison of LS means for each Bio-E-Gel treatment group with placebo (Dunnett's test).

Cross Reference: NDA Section 8, V26

2.2.2 What are the single dose and multiple dose PK parameters for estradiol?

Estradiol C_{max} and AUC_{0-24} at steady state were approximately 2-fold higher than following a single dose indicating an accumulation factor of approximately 2. The following table shows a summary of PK parameters for single and multiple dose of Bio-E-Gel.

Table 6: Unadjusted estradiol PK parameters for all doses examined

Single dose PK (Day 1)[a]							
Dose g/day	AUC_{0-24} (pg*h/mL)	C_{max} (pg/mL)	C_{ave} (pg/mL)	C_{min} (pg/mL)	T_{max}[b] (h)		
.87[c]	179.0±113.0	13.0±6.4	8.2±4.2	2.9±3.9	18 (2-20)		
1.7[c]	421.9±296.3	31.4±22.9	17.6±12.3	4.7±8.2	20 (4-24)		
2.6[d]	ND	ND	ND	ND	ND		
1.25[e]	275±172	23±16	11.5	NC	20 (2-24)		
2.5[e]	497±481	37±27	20.7	NC	16 (12-24)		
Multiple dose, steady state PK[a] (Day 14 or 15)							
Dose g/day	AUC_{0-24} (pg*h/mL)	C_{max} (pg/mL)	C_{ave} (pg/mL)	C_{min} (pg/mL)	T_{max}[b] (h)	Fluctuation index[f]	E2:E1 ratio[g]
.87[c]	335.2±166.0	21.6±13.7	15.4±5.4	9.4±2.9	18 (1-20)	0.80	0.53
1.7[c]	940.2±623.8	66.7±38.3	39.2±26.0	21.1±15.0	4 (1-20)	1.16	0.98
2.6[d]	1783.6 ± 939.9	157.8±107.9	74.3±39.2	NC	4 (2-4)	NC	1.30
1.25[e]	568±302	36±24	23.7±12.6	15±7	13 (1-24)	0.89	0.52
2.5[e]	1282±500	88±48	53.4±20.8	31±11	22 (2-24)	1.07	0.71

[a] mean ± SD unless indicated otherwise; [b] Median and range; [c] from EST007; [d] from EST008 Day 15 data; [e] from EST003; ND = no data. NC = not calculated. [f] Fluctuation index = (mean C_{max} – mean C_{min})/mean C_{ave}. [g] E2:E1 ratio was calculated based on the C_{ave} values at steady state on Day 14. Shaded areas are from application to the thigh.

2.2.3 What is the estradiol exposure following Bio-E-Gel application relative to that of other approved estradiol topical products?

The following table summarizes the PK parameters in the label of other approved estradiol products (rounded to the nearest integer) along with those of Bio-E-Gel for comparison. The estradiol exposure following 0.87 g/day Bio-E-Gel was the lowest with C_{ave} less than Climara and Vivelle patches labeled as 0.025 mg/day. The 1.7 g dose resulted in exposure comparable to that of Climara 0.05 mg/day, higher than Estrogel 1.25 g, but interestingly was less than Vivelle 0.0375 mg/day. The 2.6 g dose was similar to Vivelle 0.075 mg/day patch and less than the 0.1 mg patches. Estrasorb labeled as 0.05 mg/day only had trough values in its label, but the value of 70.2 pg/ml suggest its delivery was similar to patches labeled as 0.075 – 0.1 mg/day. Overall, lowest dose of Bio-E-Gel provides exposure that is the lowest of approved topical estradiol products and the highest dose results in exposures lower than the highest currently approved product.

Table 7: PK parameters for Bio-E-Gel and other topical estradiol products listed in the PDR.

Drug	Strength (mg/day or as indicated)	AUC (pg.h/ml)	C_{max} (pg/ml)	C_{min} (pg/ml)	C_{ave} (pg/mL)	C_{ave} Rank
Climara	0.025		32	17	22	2
Climara	0.05		71	29	41	6
Climara	0.1		147	60	87	10
Climara (applied to buttock)	0.1		174	71	106	13
Vivelle	0.025		46	30	34	4
Vivelle	0.0375		83	41	57	7
Vivelle	0.075		99	60	72	8
Vivelle	0.1		133	90	89	11
Vivelle (applied to buttock)	0.1		145	85	104	12
Estrasorb	0.05			70.2		
Estrogel	1.25 g		46.4		28.3	3
Vagifem tablet	0.025	563	49			
Bio-E-Gel	0.87 g	335	22	9	15	1
Bio-E-Gel	1.7 g	940	67	21	39	5
Bio-E-Gel	2.6 g	1784	158	36	74	9

2.2.4 What are the characteristics of ADME?

Bio-E-Gel rate and extent of absorption is summarized in table 6 in the above section. Note that data for the 1.25 and 2.5 g/day doses (study EST003) were from application to the front and inner thigh area. All other doses were applied to the upper arm, same as in the sponsor's proposed label.

Bio-E-Gel has an apparent long half-life (means of 55 -75 hours) that is longer than the known biological half life of estradiol. This may partly be due to a slow absorption rate from the gel or skin reservoir and endogenous estradiol production and suggests an absorption rate limited elimination.

The distribution, metabolism and excretion of estradiol are well known and have been summarized in the Guidance for Industry: Noncontraceptive Estrogen Drug Products for the Treatment of Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms - Recommended Prescribing Information for Health Care Providers and Patient Labeling. The following paragraphs are from the guidance.

> The distribution of exogenous estrogens is similar to that of endogenous estrogens. Estrogens are widely distributed in the body and are generally found in higher concentrations in the sex hormone target organs. Estrogens circulate in the blood largely bound to sex hormone binding globulin (SHBG) and albumin.
>
> Exogenous estrogens are metabolized in the same manner as endogenous estrogens. Circulating estrogens exist in a dynamic equilibrium of metabolic interconversions. These transformations take place mainly in the liver. Estradiol is converted reversibly to estrone,

and both can be converted to estriol, which is the major urinary metabolite. Estrogens also undergo enterohepatic recirculation via sulfate and glucuronide conjugation in the liver, biliary secretion of conjugates into the intestine, and hydrolysis in the intestine followed by reabsorption. In postmenopausal women, a significant proportion of the circulating estrogens exist as sulfate conjugates, especially estrone sulfate, which serves as a circulating reservoir for the formation of more active estrogens.

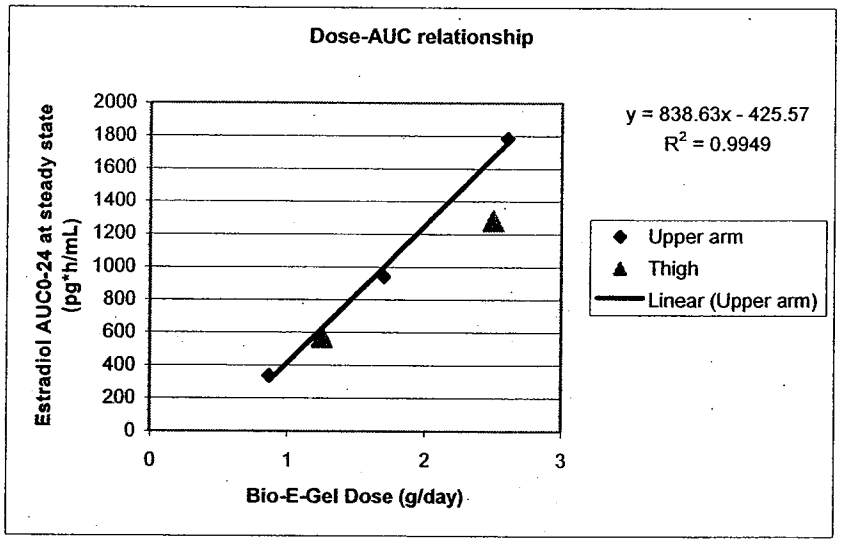
Estradiol, estrone, and estriol are excreted in the urine along with glucuronide and sulfate conjugates.

2.2.5 What is the linearity or nonlinearity of dose-concentration relationship?

Concentration increases with dose but the increase is greater than proportional. Based on studies EST007 and EST008, the mean C_{max}, C_{ave}, and AUC_{0-24} of estradiol increased with increasing daily dose from 0.87 g to 2.6 grams. The steady state exposure (AUC) appeared to increase linearly but not proportionally. There is a greater than proportional increase in exposure as dose increase. For example, AUC_{0-24} increased from 335.2 ± 166.0 pg*h/mL to 940.2 ± 623.8, a 2.8-fold increase, when dose was increased 2-fold from 0.87 g to 1.7 g. Additionally, in study EST008, where the 2.6 g dose (a 50% increase compare to the 1.7 g dose) was applied to the upper arm, the AUC_{0-24} was 1783.6 ± 939.9 pg*h/mL (pooling of both Bio-E-Gel only groups on day 15), representing a 90% increase in exposure compared to the 1.7 g dose.

Figure 7 shows the steady state estradiol AUC_{0-24} following 0.87, 1.7, and 2.6 gram/day Bio-E-Gel applied to the upper arm and the linear regression line for dose-AUC relationship. Data for 1.25 and 2.5 gram applied to the front and inner thigh are also plotted for comparison.

Figure 7: Bio-E-Gel Dose-Unadjusted estradiol AUC relationship

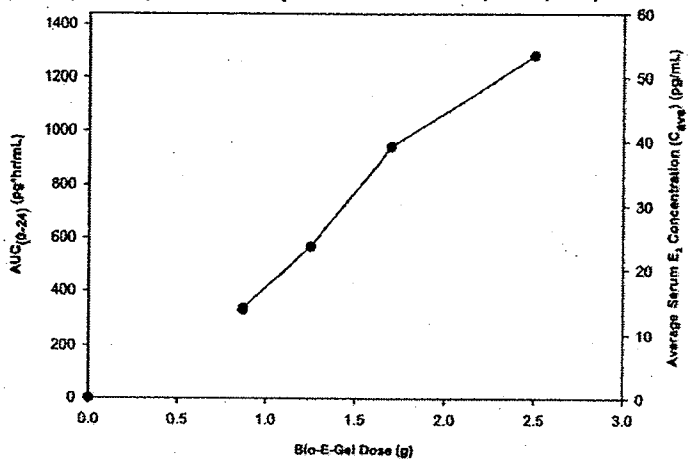


2.2.6 Does application site have an effect on drug absorption?

There is some evidence of an effect of application site on Bio-E-Gel PK, but it should not affect the acceptability of this application. The sponsor presented AUC data for dose range of 0.87 – 2.5 g/day showing increased exposure with increasing dose regardless of site (see fig. 8) and suggested that site of application may not play a major role. While this appears to be the case for

the mentioned range, the 2.6 g/day data (upper arm) showed a much greater exposure compared to the 2.5 g/day dose (thigh), indicating a potential application site effect. The different slope of the AUC vs. Dose plots for the 2 sites as shown in section 2.2.5 above also indicates a potential site effect on Bio-E-Gel's E2 exposure. Additionally, the estimated half life in subjects who applied to the thigh area was higher than those applied to the arm (see section 2.2.1), suggesting that the site of application may have affected the rate of absorption since the long half-lives in both groups are thought to be partly due to slow absorption rate. Two caveats are that these are cross study comparisons and estradiol levels are highly variable.

Figure 8: sponsor's plot of average serum E2 concentrations (unadjusted) and AUC_{0-24} (unadjusted) after multiple doses of 0.87, 1.25, 1.7, and 2.5 g Bio-E-Gel.



This lack of clear understanding should not affect the acceptability of this NDA because the sponsor is seeking only application to the upper arm, which is the site that was used in the phase 3 trial. However, comparisons of exposures at different doses from the arm and the thigh clearly show that these 2 sites cannot be concluded to result in equivalent exposure at this time. Nor is the sponsor seeking this claim in this application.

2.2.7 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Pharmacokinetics of Bio-E-Gel has been evaluated at the same doses (0.87, 1.7, and 2.6 g/day), administration schedule (once daily), and site of application (upper arm) as the phase 3 trial (EST005). In phase 1 studies, E2, E1, and E1-S concentrations were examined for 24 hours after the first dose (for 0.87 g and 1.7 g doses) and at steady state for all 3 doses (Day 14 or 15). The PK analysis showed that estradiol levels increased following Bio-E-Gel application and the ratio of estradiol:estrone approached 1 with the 1.7 g/day dose. This ratio further increases to 1.3 with the 2.6 g/day dose (EST008). The proposed [ ] administration are identical to that tested in the phase 3 trial EST005. Additionally, pre-dose sampling done in study EST005 at week 4, week 8, and week 12 indicated Bio-E-Gel was bioavailable in the systemic circulation following topical application.

2.2.8 Are the active moieties in the plasma (or other biological fluids) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationship?

Yes. Serum concentrations of estradiol and its metabolites, estrone and estrone sulfate, were characterized following Bio-E-Gel application. A summary of assays used and method validations can be found in section 2.5: General Biopharmaceutics.

2.2.9 What are the characteristics of the dose-response relationship for efficacy?

Assessment of dose response was evaluated based mainly on Phase 3 trial EST005. Phase 2 trial EST004, a dose finding study of only 4 weeks duration, provided supporting safety data.

Vasomotor Symptoms:

The currently FDA recommended 2 co-primary endpoints for VMS are 1) reduction in moderate-to-severe hot flush frequency and 2) reduction in moderate-to-severe hot flush severity at week 4 and 12. The primary endpoints should show statistical significant and in the case of frequency show a clinically significant reduction in frequency of at least two moderate to severe hot flushes above placebo.

Table 8 shows the mean changes at the primary endpoints of week 4 and week 12. Tables 9 and 10 show changes at all weekly intervals. The mean changes in daily moderate-to-severe hot flush rate or severity increased with time until apparent plateaus were reached in all groups, including placebo. The 2 higher doses of 1.7 and 2.6 g/day had statistically significant changes at both week 4 and week 12. The lowest dose of 0.87 g/day did not meet statistical significance at week 4 but did so at week 5 and week 12.

There appears to be a positive dose-response relationship relative to mean change in daily moderate-to-severe hot flush rate. Placebo, 0.87 g, 1.7 g, and 2.6 g doses reduced the daily moderate-to-severe hot flush rate by means of 5.4, 6.6, 8.2, and 9.5 at 4-week and by 6.1, 9.1, 10.7, and 11.3 at week 12, respectively (Table 8 and fig. 9).

There also appears to be a positive dose-response relationship with the reduction in daily hot flush severity (Table 8 and fig. 10).

Table 8: Mean change from baseline in daily moderate-to-severe hot flush rate and hot flush severity at week 4 and week 12 (Study EST005) (ITT-LOCF)

Evaluation	Placebo (N=137)	Bio-E-Gel 0.87 g/day (N=136)	Bio-E-Gel 1.7 g/day (N=142)	Bio-E-Gel 2.6 g/day (N=69)
	Daily Moderate-to-Severe Hot Flush Rate[a]			
Baseline (Mean ± SD)[b]	13.5 ± 4.5	13.3 ± 4.6	13.1 ± 6.5	12.9 ± 4.5
Week 4	-5.4	-6.6	-8.2***	-9.5***
Week 12	-6.1	-9.1***	-10.7***	-11.3***
	Daily Hot Flush Severity[a,c]			
Baseline (Mean ± SD)[b]	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3
Week 4	-0.3	-0.5	-0.7***	-1.0***
Week 12	-0.4	-0.9***	-1.3***	-1.6***

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

[b] Unadjusted means and standard deviations. Baseline based on the first 14 days of the Screening Period.

[c] Severity score: 0=none, 1=mild, 2=moderate, 3=severe.

SD: standard deviation

***$P<0.0001$ for treatment comparison with placebo (Dunnett's test).

Cross reference: Study EST005 (Synopsis)

Table 9: Mean change from baseline in daily moderate-to-severe hot flush rate

Evaluation	Mean Change From Baseline[a,b]			
	Bio-E-Gel 0.87 gram/day N = 137	Bio-E-Gel 1.7 gram/day N = 142	Bio-E-Gel 2.6 gram/day N = 69	Placebo N = 137
Baseline (Mean ± SD)[c]	**13.3 ± 4.6**	**13.1 ± 6.5**	**12.9 ± 4.5**	**13.5 ± 4.5**
Placebo Lead-In	-2.6	-2.7	-2.7	-3.0
Week 1	-3.4	-2.3	-3.0	-4.0
Week 2	-4.6	-4.7	-5.8	-4.7
Week 3	-5.7	-6.9	-8.4***	-5.2
Week 4	**-6.6**	**-8.2*** **	**-9.5*** **	**-5.4**
Week 5	**-7.7*** **	-9.0*** **	-10.0*** **	-5.5
Week 6	-7.9*** **	-9.5*** **	-10.4*** **	-5.7
Week 7	-8.5*** **	-9.9*** **	-10.9*** **	-6.0
Week 8	-8.6*** **	-10.1*** **	-11.0*** **	-6.0
Week 9	-8.7*** **	-10.3*** **	-1104*** **	-6.0
Week 10	-9.0*** **	-10.5*** **	-11.3*** **	-6.0
Week 11	-9.0*** **	-10.5*** **	-11.3*** **	-6.1
Week 12	**-9.1*** **	**-10.7*** **	**-11.3*** **	**-6.1**

Source: Adapted by *Dr. Theresa H. van der Vlugt* from NDA 21-813/S-000, Table 11.4-1, Section 8, Volume 26, page 83 (page 103 of 369).

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

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

* P<0.01 for treatment comparison with placebo (Dunnett's test).

** P<0.001 for treatment comparison with placebo (Dunnett's test).

*** P<0.0001for treatment comparison with placebo (Dunnett's test).

Table 10: Mean change from baseline in hot flush severity

Evaluation	Mean Change From Baseline[a,b]			
	Bio-E-Gel 0.87 gram/day N = 137	Bio-E-Gel 1.7 gram/day N = 142	Bio-E-Gel 2.6 gram/day N = 69	Placebo N = 137
Baseline (Mean ± SD)[c]	**2.4 ± 0.3**	**2.4 ± 0.3**	**2.4 ± 0.3**	**2.4 ± 0.3**
Placebo Lead-In	-0.1	-0.1	-0.1	-0.1
Week 1	-0.2	-0.1	-0.1	-0.2
Week 2	-0.2	-0.3	-0.4†	-0.2
Week 3	-0.3	-0.5***	-0.7***	-0.3
Week 4	**-0.5**	**-0.7*** **	**-0.1**	**-0.3**
Week 5	**-0.6*** **	-0.8*** **	-1.1*** **	-0.3
Week 6	-0.6*** **	-0.9*** **	-1.2*** **	-0.3
Week 7	-0.7*** **	-1.0*** **	-1.3*** **	-0.3
Week 8	-0.7*** **	-1.1*** **	-1.4*** **	-0.3
Week 9	-0.8*** **	-1.1*** **	-1.5*** **	-0.3
Week 10	-0.8*** **	-1.2*** **	-1.6*** **	-0.3
Week 11	-0.9*** **	-1.2*** **	-1.5*** **	-0.3
Week 12	**-0.9*** **	**-1.3*** **	**-1.6*** **	**-0.4**

Source: Adapted by *Dr. Theresa H. van der Vlugt* from NDA 21-813/S-000, Table 11.4-2, Section 8, Volume 26, page 86 (page 105 of 369).

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

b. Severity score: 0=none, 1=mild, 2=moderate, 3=severe.

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

† p<0.05 for treatment comparison with placebo (Dunnett's test).

* P<0.01 for treatment comparison with placebo (Dunnett's test).

** P<0.001 for treatment comparison with placebo (Dunnett's test).
*** P<0.0001 for treatment comparison with placebo (Dunnett's test).

Figure 9: Dose-reduction on hot flush rate relationship

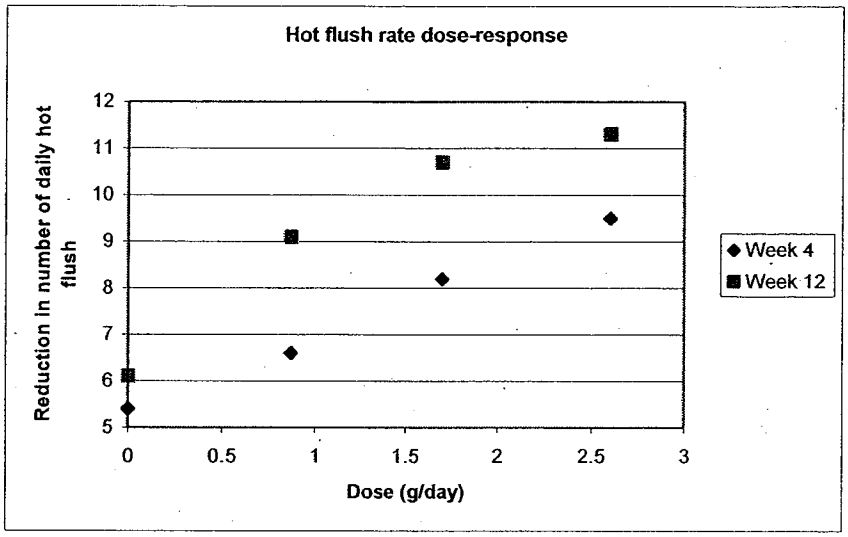
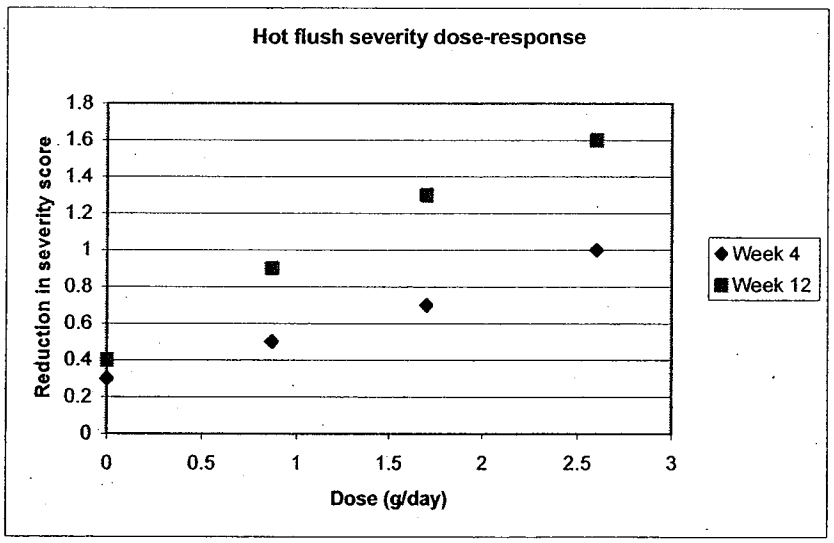


Figure 10: Dose-reduction in hot flush severity relationship



Vulvar and Vaginal Atrophy:

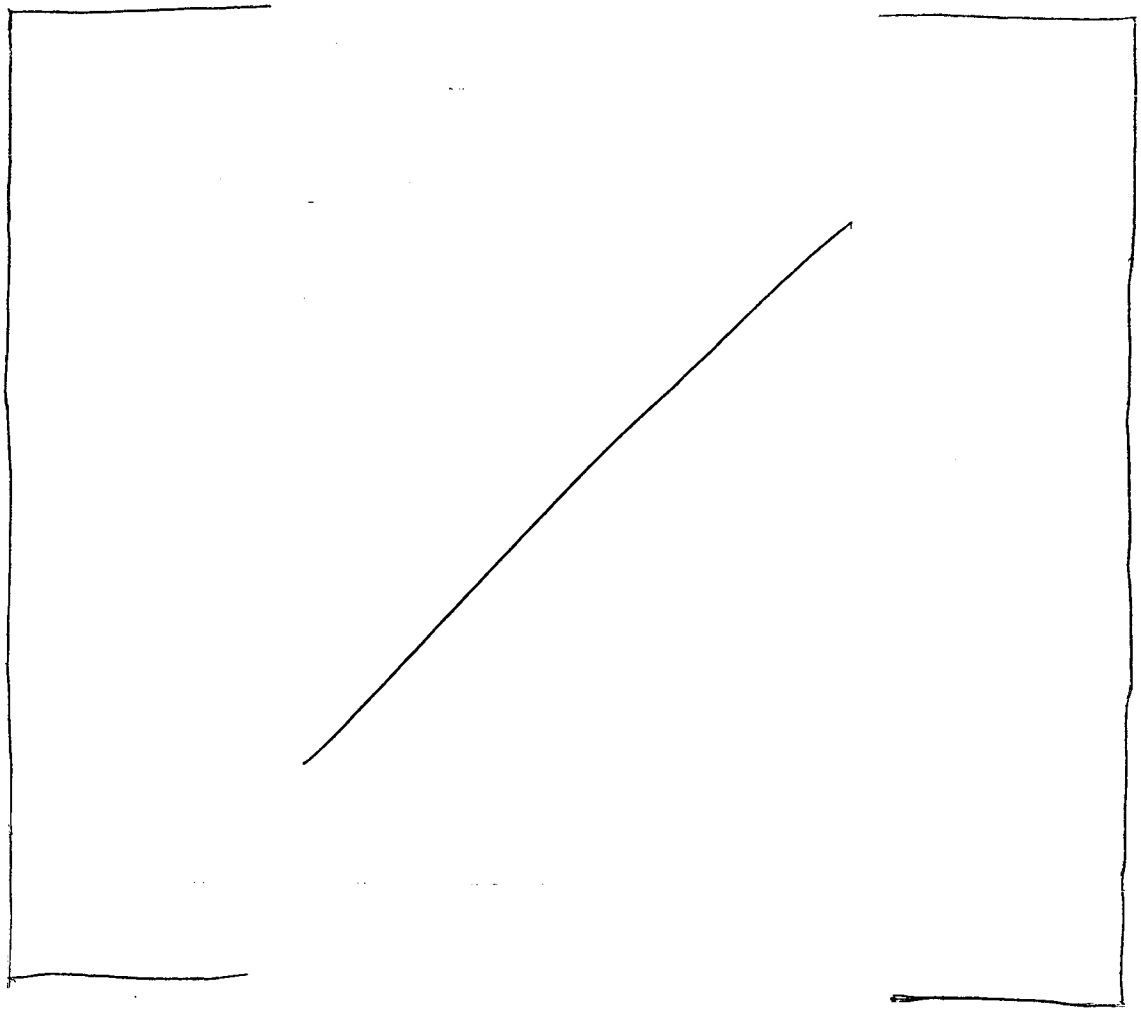
The currently FDA recommended primary endpoints for VVA includes 3 co-primary endpoints listed below.

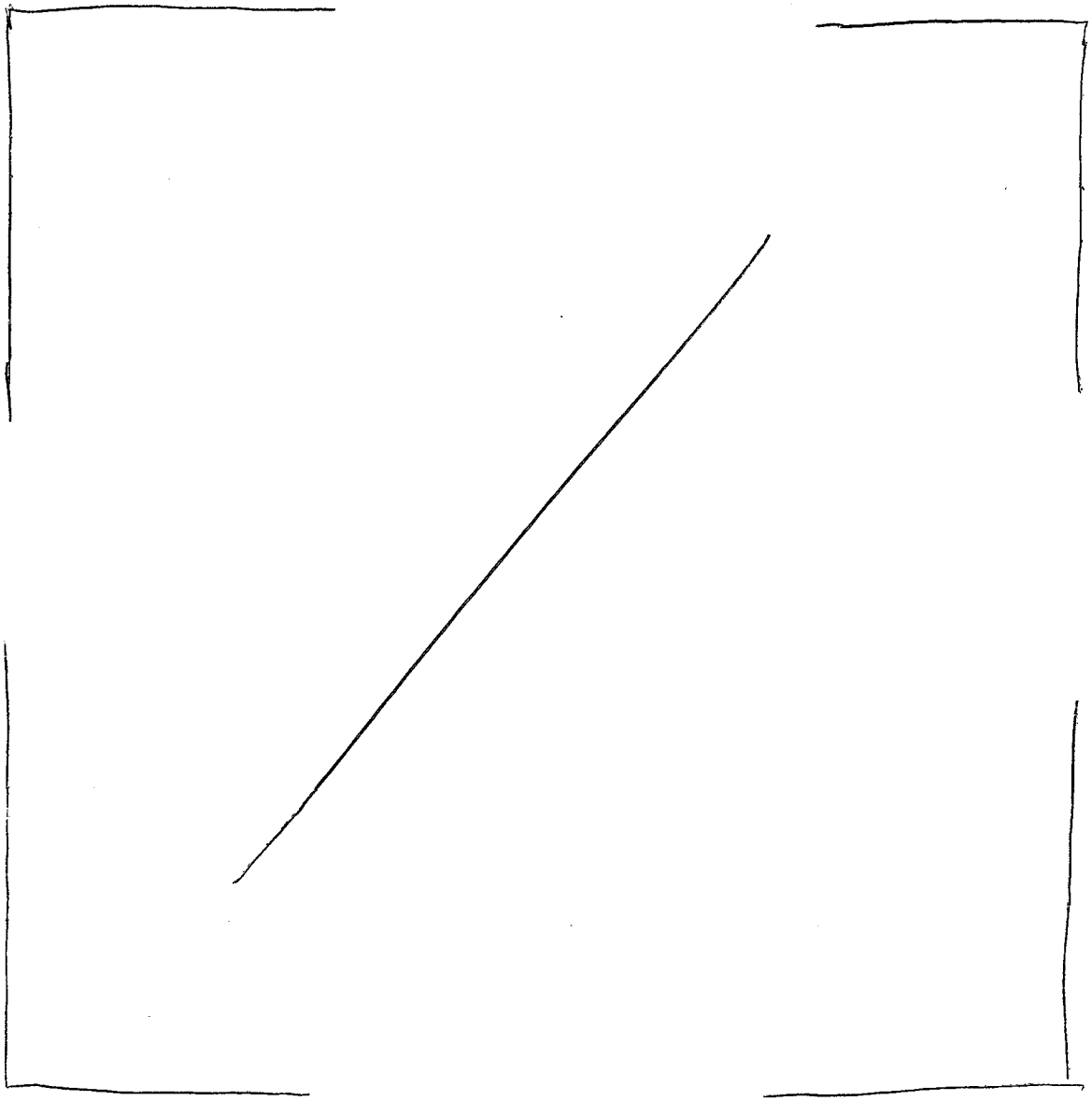
1. The mean change from baseline to week 12 in the vaginal maturation index (superficial and parabasal cells). For study inclusion, study participants would have no greater than 5 percent superficial cells on a vaginal smear at baseline. The primary efficacy analysis should show a statistically significant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

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

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

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





In summary, there were apparent positive dose-response relationships for both co-primary endpoints for VMS. [redacted]

2.2.10 What are the characteristics of the dose-response relationship for safety?

The main data used to assess exposure-safety response was trial ES005.

Significant adverse events:

In study EST005, there were 6 cases of endometrial hyperplasia reported. They were: one case of complex hyperplasia with atypia in the Bio-E-Gel 1.7 gram per day treatment group (incidence rate of 1.05%, one case per 95 subjects with a uterus) and five cases of simple hyperplasia in the Bio-E-Gel 2.6 gram per day treatment group (incidence rate of 11.1%, five cases per 45 subjects

with a uterus). None were reported for the 0.87 g/day dose or placebo. The higher rate of endometrial hyperplasia in the highest dose of 2.6 g/day represents a significant safety risk[ ]

Common adverse events:
As shown in table 14, the most common adverse events are related to reproductive system and breast. There was also an apparent dose dependent increase in frequency of adverse events in this class. The rate increased from 9.5% for placebo to 15.4, 28.9, and 46.5% for 0.87, 1.7, and 2.6 g/day doses, respectively. The only other system organ class that showed a positive dose-adverse event relationship was the nervous system, where there were adverse event rates of 5.1, 8.5, and 10.1% for 0.87, 1.7, and 2.6 g/day doses, respectively while the placebo rate was 6.6%. Other common classes of adverse events were evenly distributed among the dose groups.

Table 14: Common adverse event

System Organ Class[a]	Number (%) of Subjects			
	Bio-E-Gel 0.87 gram/day (N = 136)	Bio-E-Gel 1.7 gram/day (N = 142)	Bio-E-Gel 2.6 gram/day (n = 69)	Placebo (N = 137)
Any adverse event	80 (58.8)	92 (64.8)	47 (68.1)	77 (56.2)
Blood and lymphatic systems	1 (0.7)	0 (0)	2 (2.9)	1 (0.7)
Cardiac disorders	1 (0.7)	0 (0)	0 (0)	2 (1.5)
Ear and labyrinth disorders	0 (0)	2 (1.4)	0 (0)	1 (0.7)
Endocrine disorders	1 (0.7)	0 (0)	0 (0)	0 (0)
Eye disorders	0 (0)	0 (0)	2 (2.9)	1 (0.7)
Gastrointestinal disorders	19 (14.0)	16 (11.3)	9 (13.0)	9 (6.6)
General disorders and administration site conditions	15 (11.0)	18 (12.7)	9 (13.0)	12 (8.8)
Hepatobiliary disorders	0 (0)	0 (0)	1 (1.4)	0 (0)
Immune system disorders	1 (0.7)	0 (0)	0 (0)	3 (2.2)
Infections and infestations	2 (1.5)	4 (2.8)	1 (1.4)	2 (1.5)
Injury, poisoning and procedural complications	1 (0.7)	5 (3.5)	2 (2.9)	4 (2.9)
Investigations	4 (2.9)	5 (3.5)	3 (4.3)	4 (2.9)
Metabolism and nutrition disorders	3 (2.2)	1 (0.7)	1 (1.4)	0 (0)
Musculoskeletal and connective tissue disorders	17 (12.5)	11 (7.7)	8 (11.6)	15 (10.9)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	1 (0.7)	1(0.7)	1 (0.7)	0 (0)
Nervous system disorders	7 (5.1)	12 (8.5)	7 (10.1)	9 (6.6)
Psychiatric disorders	0 (0)	4 (2.8)	3 (4.3)	1 (0.7)
Renal and urinary disorders	5 (3.7)	3 (2.1)	2 (2.9)	6 (4.4)
Reproductive system and breast disorders	21 (15.4)	41 (28.9)	32 (46.4)	13 (9.5)
Respiratory, thoracic and Mediastinal disorders	34 (25.0)	29 (20.4)	10 (14.5)	27 (19.7)
Skin and subcutaneous tissue disorders	10 (7.4)	8 (5.6)	4 (5.8)	9 (6.6)
Surgical and medical procedures	0 (0)	0 (0)	0 (0)	3 (2.2)
Vascular disorders	0 (0)	0 (0)	0 (0)	2 (1.5)

Source: Adapted by *Dr. Theresa H. van der Vlugt* from NDA 21-813/S-000 Section 8, Volume 26, Table 12.2-1, page 119 (page 139 of 369).

a. A subject with more than one event represented by a given system organ class is counted only once for that system organ class.

2.2.11 What is the exposure to estradiol following direct contact with a partner?

Skin-to-skin contact (vigorous arm-to-arm contact where the gel was applied) with a male partner for 5 minutes at 2 and 8 hour post Bio-E-Gel application was studied in EST006.

No significant changes in E2 serum concentration was observed in male subjects following skin-to-skin drug contact. Post skin-to-skin drug contact (Day 1) E2 concentrations in male partners were similar to and followed a similar circadian pattern as the day before drug contact (Day -1). Exposure of E2 (described as AUC_{0-24}, C_{max} and C_{ave}) was not significantly different between Day

-1 and Day 1. No differences in exposures between groups were observed when Day -1 and Day 1. PK parameters were calculated based on adjustment of concentrations for baseline using the 0 h concentration for the respective Day.

2.2.12 What is the effect of washing on residual estradiol on the skin?

The mean percent of E2 recovered from the skin at 2 and 8 h post-application was 4.6 ± 4.0 % and 7.8 ± 5.8 % of the applied dose of E2, respectively. Washing the application area with soap and water 8 h post-application decreased the percent of E2 recovered from the skin to approximately[ ]% of the applied dose. This suggests that washing of the application site area substantially decreased the potential for transfer of Bio-E-Gel.

Thus it appears that even though absorption of Bio-E-Gel through partner transfer was not detected in this study, residual skin estradiol was present at 2 and 8 hours post application and washing the area can reduce the amount of residual estradiol.

The effect of washing on the absorption of Bio-E-Gel was not examined. However, the low residual E2 level at 2 hour post application suggests that washing the application site at 2 hour post application should not significantly affect Bio-E-Gel absorption. Therefore, it is recommended that patients wait at least 2 hours before washing.

2.2.13 Is the calculation of in vivo delivery rate acceptable?

Sponsor used the same calculation method that was applied in NDA 21-367 (Estradiol Acetate Vaginal Ring) and was found acceptable by the Office of Clinical Pharmacology. The nominal delivery rate was calculated using the following equation:

Nominal delivery (mg/24 h) = CL (1280 L/d) x Cave (pg/mL) x 1000 mL/L x 1 mg/10^9 pg (Eq. 1)

The following table shows the summary of apparent in vivo delivery rates following application of Bio-E-Gel:

Table 15: Estradiol in vivo delivery rate estimates

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
EST003[a]	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), Bio-E-Gel was applied to the front and inner thigh instead of the upper arms that was used in all other studies.

Study EST008 was a sunscreen effect study. The data included in the above table are from day 15 Bio-E-Gel only control group where 2.6 g Bio-E-Gel was applied to the upper arm once daily for 15 days.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence response and what is the impact of any differences in exposure on efficacy or safety response?

Bio-E-Gel was investigated only in postmenopausal women with normal hepatic and renal function. No attempt was made to evaluate the effect of factors such as age and hepatic and renal impairment on Bio-E-Gel PK in this NDA.

2.4 Extrinsic Factors

2.4.1 What drug interactions may affect the PK of Bio-E-Gel?

Sponsor did not conduct drug interaction studies. However, estrogen is known to be partially metabolized by CYP3A4. Therefore inducer and inhibitor of CYP3A4 may affect the metabolism of Bio-E-Gel. FDA Guidance for Industry: Noncontraceptive Estrogen Drug Products for the Treatment of Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms - Recommended Prescribing Information for Health Care Providers and Patient Labeling recommend the following to be included in the label.

> 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 What acute effect does sunscreen application has on the absorption of Bio-E-Gel?

Sponsor submitted study EST008 that examined the effect of sunscreen application 10 minutes prior or 25 minutes after Bio-E-Gel application on estradiol serum concentration. This was a 2-group, cross over study where Bio-E-Gel 2.6 g/day was applied daily for 15 days to establish steady state level for Bio-E-Gel alone. This was immediately followed by 7 daily applications (Day 16 – 22) where Bio-E-Gel was applied 10 minutes after or 25 minute before application of sunscreen. Concentration profile at the end of concomitant sunscreen application period (i.e., Day 22) was used to determine the effect of sunscreen. The cross over study continues with Bio-E-Gel only on days 23 – 37 and Bio-E-Gel with sunscreen on days 38 – 44. A more detailed review of EST008 can be found in the appendix section.

When the sunscreen ([redacted](SPF 30 UV A,UVB) sunscreen lotion) was applied 10 min before the application of Bio-E-Gel, there were increases in E2, E1, and E1-S average serum concentrations of approximately 55%, 34% and 36%, respectively. When sunscreen was applied 25 min after Bio-E-Gel application, there were no clinically significant changes in the E2, El, and E1S average serum concentrations. The mechanism of this interaction is not known.

2.4.3 Does prolonged application of sunscreen (e.g., daily for 7 days) have an effect on subsequent applications of Bio-E-Gel?

This question was raised since it was also noted in study EST008 that exposure (AUC_{0-24}) to E2 in Bio-E-Gel only subjects was [redacted]-fold higher on Day 37 compared to Day 15, where sunscreen was also applied on days 16 – 22, either before or after Bio-E-Gel application. It is not clear why there is this significant increase on Day 37 relative to Day 15.

Estradiol has been shown to cause an increase in level of sex hormone binding globulins (SHBG) that may lead to increased total serum estradiol due to its binding to SHBG. However, transdermal application of estradiol has been generally reported to not significantly affect SHBG level. Additionally, in this NDA, study EST003 showed that SHBG level only increased slightly from a baseline level of 72.5 nM to 77.83 and 88.83 nM after 7 and 14 daily applications of 2.5 g/day Bio-E-Gel. Similarly, study EST005 showed that circulating SHBG serum concentration were increased slightly compared to placebo in the Bio-E-Gel 0.87 g/day and 1.7 g/day dose groups and were statistically significantly increased in the Bio-E-Gel 2.6 g/day dose group beginning at week 4. However, all mean trough levels of SHBG (85 – 111 nM) were similar to placebo group (~80nM) and fell within the normal range (40 – 120 nM). This reviewer agrees with the sponsor's calculation that SHBG level could increase by approximately 15% from day 15 to 37 while on Bio-E-Gel. This indicates that SHBG can account for ≤ 15% of the observed 110% increase in E2 AUC_{0-24}.

Could a 3 weeks time between day 15 and Day 37 caused a shift in baseline and led to the apparent period effect? Sponsor proposed this could be the case. This reviewer does not concur since the phase 3 study EST005 showed that E2 trough levels were similar between week 4, 8, and 12 – a more prolonged period than the 3 weeks in EST008. This data suggest that there is no period effect when Bio-E-Gel is applied without sunscreen.

Sponsor also proposed several other explanations for the increase exposure in the second period:

- The technician applying Bio-E-Gel in EST008 may have improved skills over the period of time and that there are 2 addition steps (e.g., weighing and transfer to the technician gloved hand before application) that represents potential source of error and possible improvement over time. While this is possible, it is unlikely as the technicians are trained and the procedure is not complicated (pump, weigh to make sure pumping was accurate, and transfer to site of application and spread). Furthermore, the extra step of weighing is meant to increase consistency among applications.
- Sponsor commented that it is reassuring that trough E2 levels in EST005 were similar at 4, 8 and 12 week. This reviewer agrees; however since study EST005 did not include sunscreen application it does not help explain what will occur in patients that use sunscreen.
- Sponsor noted that literature reports indicate that sunscreen absorption is negligible and chance of interaction between Bio-E-Gel and sunscreen applied a week earlier. However, an ideal sunscreen would impregnate the stratum corneum and create a UV protective layer. This possibly may alter the top layer of skin to allow better absorption of Bio-E-Gel, which is suggested by the effect of sunscreen application prior to Bio-E-Gel. It is also theoretically possible that this effect is greater with multiple applications and E2 accumulates over time.
- The increase on Day 37 may be due to very high AUC by subject 103 (AUC for subject 103 was 10202 pg*h/mL while the mean for the entire group was 3670 pg*h/mL). However, even if this subject is removed, which is not appropriate, the ratio of mean AUC_{0-24} on Day 37 and Day 15 for Bio-E-Gel alone was still 1.7 or 70% increase.

Could the 1 week application of sunscreen affect the absorption of Bio-E-Gel during the 2 weeks period following discontinuation of sunscreen? Of the possible explanations by FDA and sponsor, the only feasible one is SHBG, which only can account for up to about 15% of the increase. The period effect due to Bio-E-Gel alone and technical application improvement over time is possible but not likely. By deduction, it appears that the 7 days of sunscreen application on days 16 – 22 may have contributed a majority of the increase in Bio-E-Gel bioavailability on day 37. The mechanism of this proposed phenomenon is not known.

Based on these results, it is recommended that occasional sunscreen users should not apply sunscreen to the same site prior to Bio-E-Gel application and only apply sunscreen at least 25 minutes after Bio-E-Gel application. Daily application of sunscreen to site of Bio-E-Gel application

may lead to increased estradiol serum concentrations following Bio-E-Gel application even if sunscreen use is discontinued.

2.5 General Biopharmaceutics

2.5.1 Is the to-be-marketed formulation identical to the one used for the phase 3 efficacy trials?

Yes.

2.5.2 What is the formulation?

The table below shows the proposed commercial formulation for Bio-E-Gel. This formulation is the same one used in phase 2 and 3 clinical studies and all phase 1 studies that are being used to support the NDA.

Table 16: Final formulation of Bio-E-Gel

Component	% w/w
Estradiol, USP	0.06%
Ethanol, [ ]	
Propylene glycol	
Diethylene glycol monoethyl ether [ ]	
[ ] (Carbomer® 940)	
Triethanolamine [ ]	
Purified water	
Edetate disodium	

2.5.3 What are the proposed in vitro release rate method and specification and how do these assure in vivo performance and quality of the product?

The in vitro release specifications are reviewed by ONDQA. Please refer to Dr. Zhengfang Ge's review.

Note: The in vitro release test has not been validated to predict in vivo performance. Additionally, the in vitro release testing, alone, is not a surrogate test for in vivo bioavailability or bioequivalence, according to FDA Guidance for Industry SUPAC-SS (1997).

2.5.4 Is drug delivery similar between the metered dose pump and [ ]?

[ ]

Specifications for the pumps are

Single actuation	0.87 g / %
Mean weight of 10 actuations	0.87 g / %
	With NMT /% of single actuation can be outside this range.

[ ]

2.6 Analytical Section

2.6.1 What bioanalytical methods were used to assess concentrations?

1) A radioimmunoassay assay (RIA) was used to quantitative assessment of estradiol, estrone, and estrone sulfate in serum.

Estradiol: After liquid extraction and [ ] chromatography, estradiol was measured using a radioimmunoassay with a specific anti-estradiol antibody. The anti-estradiol antibody did not cross react with a panel of estrogens and steroids with the exception of estrone (1.3%) and estriol (0.6%). The combination of specific antibody and [ ] chromatography allows for a sensitive assay and reduces cross-reactivity. Recovery is monitored for each individual sample via spiking of tritium labeled estradiol and is taken into account during calculation.

Estrone: Estrone samples are also subjected to extraction and [ ] chromatography before RIA. The antiserum for estrone significantly cross-reacts with estradiol (77%). This was overcome by effective chromatographic separation. The antiserum for estrone also cross reacted with other steroids at level of 3.5% or less. Individual recovery was monitored via spiked tritiated estrone and used during calculation.

Estrone sulfate: Estrone sulfate was measured as estrone by above radioimmunoassay following enzymatic hydrolysis of estrone sulfate into estrone by a 16-hour incubation with sulfatase at 42 degree Celsius. Individual recovery was monitored using tritiated estrone sulfate.

Sex hormone binding globulin (SHBG): SHBG was measured by immunoradiometric assay (IRMA).

2) An LC/MS/MS method was used to assess estradiol concentration in the swabs that were used in study EST006. [ ] was used as the internal standard. The assay has a range of 0.50 to 500 ng/mL and 400 to 200,000 ng/mL.

2.6.2 Are the analytical assay methods adequately validated?

Yes. Assays for estradiol, estrone, estrone sulfate, and SHBG were validated assays.

Estradiol assay has a lower limit of quantitation (LOQ) of 1.0 pg/tube or 0.5 ng/dL using a 2 ml sample volume. Intra-assay variability were 13% and 6.2% at the extremes of the standard range of 0.5 ng/dL to 30 ng/dL. Inter-assay variations of assay controls were 15% at 2.6 ng/dL, 10% at 13 ng/dL, and 10% at 23 ng/dL.

Estrone assay has a LOQ of 1.0 pg/tube or 0.5 ng/dL using a 2 ml sample volume. Good precision was obtained for samples range of 0.8 ng/dL to 24 ng/dL with intra-assay variation of 13.9% at both extremes. Inter assay variations of assay controls were 15% at 2.7 ng/dL, 12% at 12 ng/dL, and 16% at 20 ng/dL.

Estrone sulfate assay has a LOQ of 2 pg/tube with 15% CV. Intra-assay CV% were 8.0 and 6.1% for 2 control pools covering high and low levels typically found in a patient population. The assay has been shown to be valid over a range of 333 – 546 ng/dL of estrone sulfate. Inter-assay CV% ranged from 11.5 to 8.2%.

Estradiol LC/MS/MS assay has a LOQ of 0.5 ng/mL with a CV of 6.64%. Intra-assay variability ranged from 2.12% to 5.16% using spiked samples at 1.50, 150.0, and 350.0 ng/mL. Inter-assay variability ranged from 1.92 to 6.18% in the concentration range of 0.50 to 500 ng/mL. The range of 400 to 200,000 ng/mL was partially validated (3 batches). The precision and accuracy were similar to the lower range.

SHBG IRMA had a typical intra-assay precision of approximately 1.2 – 5.3%. The LOQ is about 1.6 nM.

3 Detailed Labeling Recommendations

The following are general labeling recommendations:

- Sunscreen should be applied at least 25 minutes after application of Bio-E-Gel
- Avoid applying sunscreen for 7 days or more to the Bio-E-Gel application area
- Include pharmacokinetic parameters for all dose levels
- Include standard deviation bars in the estradiol plot

4 Appendices

4.1 Proposed labeling (Original and Annotated)

Please see final label in DFS if approved at time of action.

4.2 Individual Study Reviews

4.3 Cover sheet and OCPB Filing/Review Form

Please see separate file in DFS.

Appears This Way
On Original

Appendix 4.2.1: Study EST007 review

Appendix 4.2.2: Study EST003 review

Appendix 4.2.3: Study EST006 review

Appendix 4.2.4: Study ESt008 review

Study EST 007 Review

This was a phase 1, single-center, open-label, parallel group, randomized study evaluating single and multiple dose PK profiles in 24 healthy postmenopausal female subjects. Twelve subjects (Group 1) received 1.7 g/day (1.02 mg E2/day) of Bio-E-Gel and 12 subjects (Group 2) received 0.87 g/day (0.52 mg E2/day) Bio-E-Gel daily for 14 consecutive days (Days 1 – 14). The gel was applied to the same upper arm area measuring approximately 320 cm^2 once daily.

Baseline samples were taken on Day -1 (-24 h and -12 h) and Day 0 (0 h prior to dosing). On Day 1 and 14, PK samples were taken at 0 h (prior to dosing), 1, 2, 4, 6, 8, 12, 16, and 24 h post dose. The 24 h post dose samples also served as trough level for the next day. Samples for trough E2 levels were taken on Days 2-5, 7, 9, 11, 13, and 14 prior to gel administration.

Following the last dose on Day 14, samples were also drawn on Days 15 (24 h post dose), 16 (48 h), 18 (96 h), and 20 (144 h) for E2, E1, and E1-S analyses.

Serum E2, E1, and E1-S were measured using validated radioimmunoassay methods. Sample values below LOQ were set to zero.

Baseline was calculated by averaging the 3 baseline samples. For baseline adjusted PK parameters, the individual subject's baseline was subtracted from the total serum hormone levels then PK parameters were calculated from the net level. Negative net levels were set to zero.

Due to 2 dropouts (1 from each group) and 1 excluded due to missing 2 doses on Days 12 and 13 (subject 108, 1.7 g group), there are data from 10 subjects from Group 1 (1.7 g) and 11 subjects from Group 2 (0.87 g) for PK analysis.

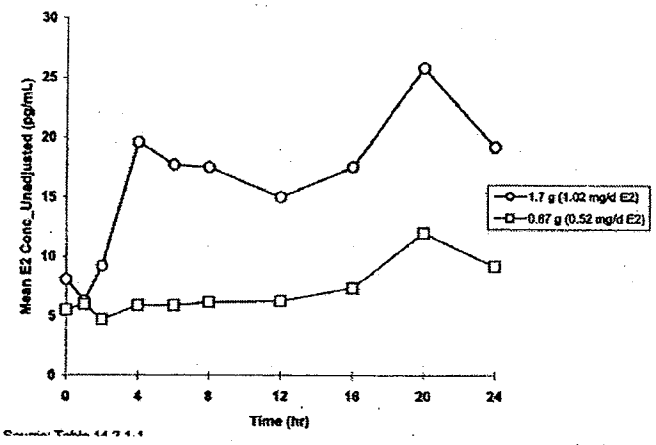
Results:

Estradiol:

First dose PK:

Following a single application of 1.7 g ofBio-E-Ge1, mean serum E2 concentrations increased from a baseline value of 8.1 ± 8.86 pg/mL and reached levels of 25.8 ± 15.5 pg/mL at 20 h. For the 0.87 g dose, mean serum E2 concentrations increased from a baseline value of 5.6 ± 5.72 pg/mL and reached levels of 12.0 ± 7.14 pg/mL at 20 h. Mean unadjusted serum E2 concentrations increased from baseline within the first 2-4 h following a single application of 1.7 g or 0.87 g of Bio-E-Gel. The figure below show the mean E2 concentration after first dose.

Figure 11: Mean unadjusted serum E2 concentrations after a single dose of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel.



Trough levels (Day 1 – Day 14):

Following repeated daily applications of 1. 7 g of Bio-E-Gel, mean serum trough E2 concentrations increased rapidly from a Day 1, pre-dose value of 8.1 ± 8.86 pg/mL. The trough level at Day 2 rose to 19.2 ± 12.78 pg/mL and a value of 26.4 ± 14.6 pg/mL was reached on Day 14. Sponsor suggested that the upward trend at Day 14 was likely due to data variability and outlier values from subject 104 and 109. Trough E2 concentrations varied widely from 0 pg/mL to a high of 86.0 pg/mL over the 14-day sampling period.

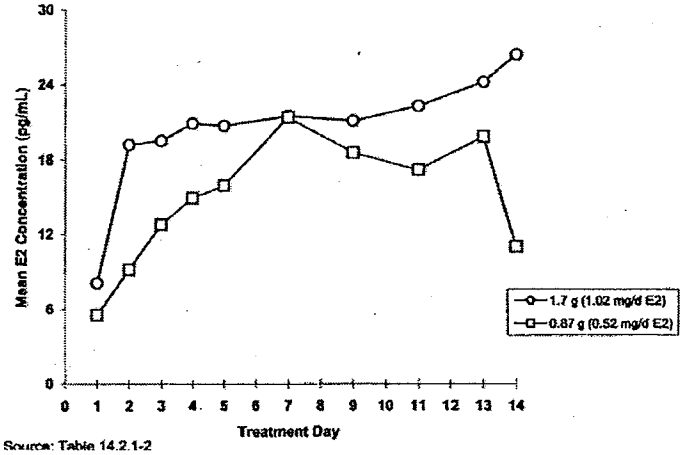
The sponsor indicated that the time to steady-state was estimated by visually examining the median data and performing a regression analysis of the mean data. The median data indicated that a plateau had been reached at the end of Day 2; the regression analyses calculated that steady-state E2 concentrations were reached at Day 3. Following the last application of 1.7 g of Bio-E-Gel on Day 14, mean serum E2 concentrations declined to 11.20 ± 20.08 pg/mL by 96 h post dose, which was similar to the pre-dose baseline levels.

Following repeated daily applications of 0.87 g of Bio-E-Gel, mean serum trough E2 concentrations increased from a Day 1, pre-dose value of 5.55 ± 5.72 pg/mL. The trough levels between Days 2 and 7 increased moderately until a plateau level of 21.36 ± 11.43 pg/mL was reached. The trough E2 value at Day 14 was 11.0 ± 5.9 pg/mL. Although the concentration time curve for trough levels appeared to be trending downward at Day 14, the effect was likely from the variability observed in the mean data and outlier values in subject 202. Trough E2 concentration data over the 14-day sampling period varied from a low of 0.0 pg/mL to a high of 54.0 pg/mL.

Time to reach steady-state E2 trough concentrations was again estimated by visually examining the median data and performing a regression analysis of the mean data. The median data suggested that a plateau had been reached by Days 3 or 4; the regression analyses calculated that steady-state E2 concentrations were reached at Day 3. Following the last application of 0.87 g Bio-E-Gel on Day 14, mean serum E2 concentrations declined to 7.27 ± 5.35 pg/mL by 96 h post dose, which was similar to the pre-dose baseline levels.

The figure below shows the mean E2 trough levels over the 14-day sampling period.

Figure 12: Mean unadjusted trough serum E2 concentrations of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 1-14).

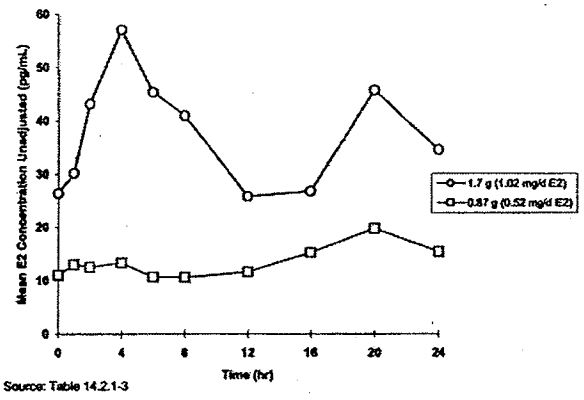


Source: Table 14.2.1-2

Day 14 PK:

The figure below show the E2 profiles for 24 hour post dose on Day 14. The mean E2 concentrations at the beginning (1.7 g: 26.4 pg/mL; 0.87 g: 11.0 pg/mL) and end (1.7 g: 34.6 pg/mL; 0.87 g: 15.5 pg/mL) of this 24 h sampling interval were comparable. There was large fluctuation in mean concentration for the 1.7 g dose.

Figure 13: Mean unadjusted serum E2 concentrations after multiple doses of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 14).



Source: Table 14.2.1-3

Comparison of Day 1 and Day 14 PK:

After a single 1.7 g dose, the median time to reach maximum concentration (Tmax) was achieved at 20 h (range: 4 h - 24 h) indicating a prolonged absorption phase. Following repeated applications the maximum concentration (Cmax) on Day 14 was approximately 2 times greater than after a single dose. The median T max estimate was approximately 4 h (range: 1 h - 20 h) on Day 14 and was earlier than the Tmax observed on Day 1. The exposure to E2 (AUC_{0-24}) was 940.2 ± 623.8 pg*h/mL on Day 14 and was more than double that observed on Day 1 (2.23-fold increase).

After a single dose of 0.87 g Bio-E-Gel, median Tmax was achieved at 18 h (range: 2 h - 20 h)

indicating a prolonged or delayed absorption phase. Following multiple applications the maximum concentration (Cmax) on Day 14 was slightly less than 2 times greater than after a single dose. The median T max estimate was approximately 18 h (range: 1 h - 20 h) on Day 14 and was comparable to Day 1. The exposure to E2 (AUC_{0-24}) on Day 14 was slightly less than double that observed on Day 1 (1.87 fold increase).

Following multiple applications, the unadjusted, dose-dependent E2 parameters (AUC_{0-24}, C_{max}, and C_{ave}) showed simple ratio increases of 2.5 to 3.1 times for the 1.7 g Bio-E-Gel dose as compared to the 0.87 dose. This suggests a greater than dose proportional increase.

Based on the E2 and E1 Cave values at steady-state, the E2:E1 ratios were 0.53 for the 0.87 g dose and 0.98 for the 1.7 g dose. The significance of this finding is that the 1.7 g dose corrected the E2:E1 ratio to that of a premenopausal woman (~ 1.0).

Apparent elimination half-lives from unadjusted serum E2 concentrations for 1.7 g and 0.87 g Bio-E-Gel were 112.8 ± 216.7 h and 210.1 ± 229.7 h, respectively. These half-lives are longer than the known biologic half-life of E2, indicating that the calculation of the half-life was complicated by prolonged absorption of depot E2 from the gel and/or the skin as well as by endogenous production of E2.

Table 17: Estradiol PK parameters for unadjusted serum concentrations [single dose (Day 1) and multiple dose (Day 14)]

Treatment Group	Descriptive Statistics	AUC_{t-24} (pg·h /mL)	C_{max} (pg/mL)	C_{ave} (pg/mL)	T_{max} (h)[c]
Single Dose PK Parameters (Day 1)					
1.7 g (1.02 mg/d E2)	Mean	421.9	31.4	17.6	20.0
	SD	296.3	22.9	12.3	4-24
	%CV	70.2	72.9	69.7	48.6
	GeoMean	314.6	24.4	13.4	13.4
	N	10	10	10	10
0.87 g (0.52 mg/d E2)	Mean	179.0	13.0	8.2	18.0
	SD	113.0	6.4	4.2	2-20
	%CV	63.1	48.9	51.5	54.1
	GeoMean	-	-	7.1	11.0
	N	11	11	10[b]	10[b]
Statistical comparison for between group difference[a]		0.54	0.47	0.75	NA
Multiple Dose, Steady-state PK Parameters (Day 14)					
1.7 g (1.02 mg/d E2)	Mean	940.2	66.7	39.2	4.0
	SD	623.8	38.3	26.0	1-20
	%CV	66.4	57.5	66.4	96.9
	GeoMean	782.9	56.1	32.6	5.1
	N	10	10	10	10
0.87 g (0.52 mg/d E2)	Mean	335.2	21.6	15.4	18.0
	SD	166.0	13.7	5.4	1-20
	%CV	49.5	63.3	35.2	60.4
	GeoMean	-	-	14.6	9.3
	N	11	11	10	10
Statistical comparison for between group difference[a]		0.2	0.11	0.32	NA

[a] P-values from a 2-sided t-test for between group differences. Dose-normalized values were used in statistical comparisons for dose-dependent parameters (AUC, C_{max}).
[b] Data from Subject 201 was set to missing.
[c] For T_{max}, median values (instead of means) and range (instead of SD) are indicated.
Source: Appendices 16.1.9.2, 16.4.1.11, 16.4.1.12, 16.4.1.13 and 16.4.1.14

Baseline adjusted E2 PK:

The mean E2 values from 3 baseline samples were subtracted from that individual's total E2 level to obtain net E2 values. The baseline adjusted PK parameters are calculated from net E2 values. Baseline values ranged from a mean of 0.0 to 23 pg/mL

After multiple applications of 1.7 g dose of Bio- E-Gel, the baseline adjusted exposure to E2 ($\delta AUC_{0\text{-}24,\ ss}$) was 765.0 ± 554.7 pg*h /mL on Day 14 and was more than 3 times that observed on Day 1. After multiple applications of 0.87 g dose, the ($\delta AUC_{0\text{-}24,\ ss}$) was 201.4 ± 141.5 pg*h /mL on Day 14 and was approximately 4 times that observed on Day 1.

Apparent elimination half-lives from baseline-adjusted serum E2 for 1.7 g and 0.87 g Bio-E-Gel were 75.2 ± 147 and 54.8 ± 40.3 h, respectively.

The table below shows a summary of baseline-adjusted E2 PK parameters.

Table 18: Estradiol PK parameters for adjusted serum concentrations [single dose (Day 1) and multiple dose (Day 14)]

Treatment Group	Descriptive Statistics	δAUC_{0-24} (pg-h/mL)	δC_{max} (pg/mL)	δC_{ave} (pg/mL)
Single Dose PK Parameters (Day 1)				
1.7 g (1.02 mg/d E2)	Mean	253.2	24.10	10.6
	SD	279.4	24.5	11.6
	%CV	110.4	101.5	109.6
	GeoMean	154.6	16.0	6.6
	N	10	10	10
0.87 g (0.52 mg/d E2)	Mean	57.7	7.4	2.70
	SD	53.9	6.1	2.2
	%CV	93.4	83.3	79.8
	GeoMean	-	-	2.1
	N	11	11	10
Statistical comparison for between group difference[a]		0.14	0.26	0.19
Multiple Dose, Steady-state PK Parameters (Day 14)				
1.7 g (1.02 mg/d E2)	Mean	765	59.4	31.9
	SD	554.7	37.5	23.1
	%CV	72.5	63.1	72.5
	GeoMean	612.4	48.2	25.5
	N	10	10	10
0.87 g (0.52 mg/d E2)	Mean	201.4	16.0	9.2
	SD	141.5	11.6	5.5
	%CV	70.2	72.4	59.3
	GeoMean	-	-	7.8
	N	11	11	10
Statistical comparison for between group difference[a]		0.06	0.05	0.10

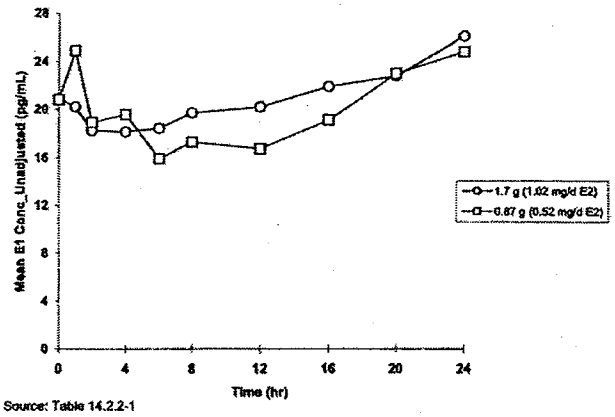
[a] P-values from 2-sided t-test for between group differences. Dose-normalized values were used in statistical comparisons for dose-dependent parameters (AUC, C_{max} and C_{ave}).

Source: Appendices 16.1.9.2, 16.4.1.11, 16.4.1.12, 16.4.1.15 and Appendix 16.4.1.16

Estrone:

The figure below shows E1 concentration profile following Day 1 dosing of Bio-E-Gel.

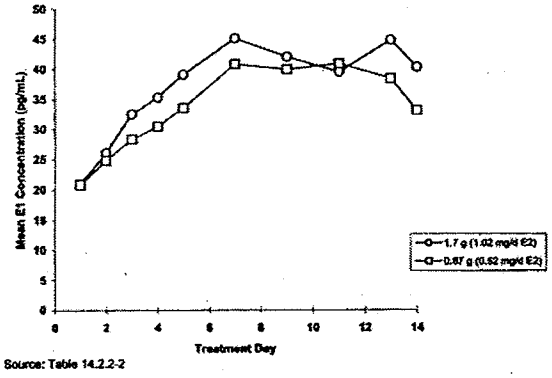
Figure 14: Mean unadjusted serum E1 concentrations after a single dose of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 1).



Source: Table 14.2.2-1

The figure below shows E1 trough concentrations over 14-day treatment with Bio-E-Gel. Estimated time to steady state was approximately 7 and 5 days for 0.87 and 1.7 g doses, respectively. Following the last application of 1.7 g of Bio-E-Ge1 on Day 14, mean serum E1 concentrations declined to 22.50 ± 13.54 pg/mL by 96 h post-dose, which was similar to the pre-dose baseline level of 20.90 ± 10.39 pg/mL. Similarly, following the last application of 0.87 g Bio-E-Gel on Day 14, mean serum E1 concentrations declined to 23.73 ± 10.87 pg/mL by 96 h post dose, which was similar to the pre-dose baseline levels of 20.8 ± 14.07 pg/mL.

Figure 15: Mean unadjusted trough serum E1 concentrations of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 1-14).



Source: Table 14.2.2-2

The figure below shows the estrone PK profile on Day 14 of Bio-E-Gel administration.

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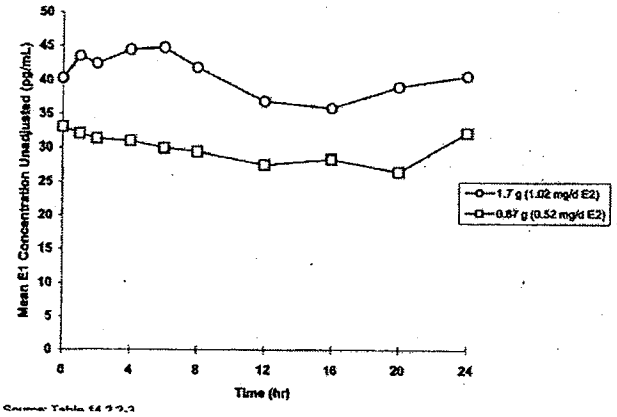
Figure 16: Mean unadjusted serum E1 concentrations after multiple doses of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 14).



Estrone exposure on day 14 was less than 2-fold higher than on day 1. Following repeated administrations, the increase in exposure to estrone was less than dose proportional. Apparent elimination half-lives from unadjusted serum E1 concentrations for 1.7 g and 0.87 g Bio-E-Gel were 137.0 ± 127.9 and 240.6 ± 179.9 h, respectively.

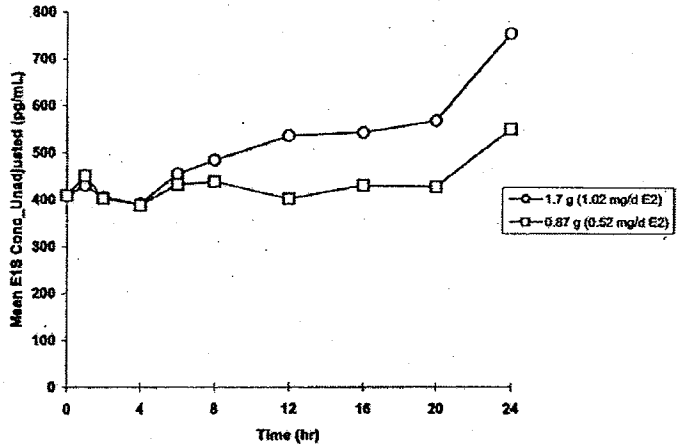
Table 19: Estrone PK parameters for unadjusted serum concentrations [single dose (Day 1) and multiple dose (Day 14)]

Treatment Group	Descriptive Statistics	$AUC_{0\text{-}24,\ ss}$ (pg·h/mL)	$C_{max,\ ss}$ (pg/mL)	$C_{ave,\ ss}$ (pg/mL)	$T_{max,\ ss}$ (h)[b]
Single Dose PK Parameters (Day 1)					
1.7 g (1.02 mg/d E2)	Mean	501.9	28.4	20.9	22
	SD	192.6	10.5	8.0	0-24
	%CV	38.4	37.0	38.4	61.9
	GeoMean	460.8	26.4	19.2	-
	N	10	10	10	10
0.87 g (0.52 mg/d E2)	Mean	471.3	27.8	19.6	2.0
	SD	231.0	9.4	9.6	0-24
	%CV	49.0	33.8	49.0	105.1
	GeoMean	412.5	26.3	17.2	-
	N	11	11	11	11
Statistical comparison for between group difference[a]		0.01	0.001	0.01	NA
Multiple Dose, Steady-state PK Parameters (Day 14)					
1.7 g (1.02 mg/d E2)	Mean	960.8	52.0	40.0	6.0
	SD	558.6	29.4	23.3	1-20
	%CV	58.1	56.5	58.1	87.7
	GeoMean	837.3	45.9	34.9	5.0
	N	10	10	10	10
0.87 g (0.52 mg/d E2)	Mean	699.1	37.8	29.2	1.0
	SD	272.6	12.9	11.3	0-24
	%CV	39.0	34.2	38.5	151.6
	GeoMean	632.5	35.4	26.5	-
	N	11	11	11	11
Statistical comparison for between group difference[a]		0.10	0.08	0.10	NA

[a] P-values from 2-sided t-test for between group differences. Dose-normalized values were used in statistical comparisons for dose-dependent parameters (AUC, C_{max}).
[b] For T_{max}, median values (instead of means) and range (instead of SD) are indicated
Source: Appendices 16.1.9.2, 16.4.2.11, 16.4.2.12, 16.4.2.13 and 16.4.2.14

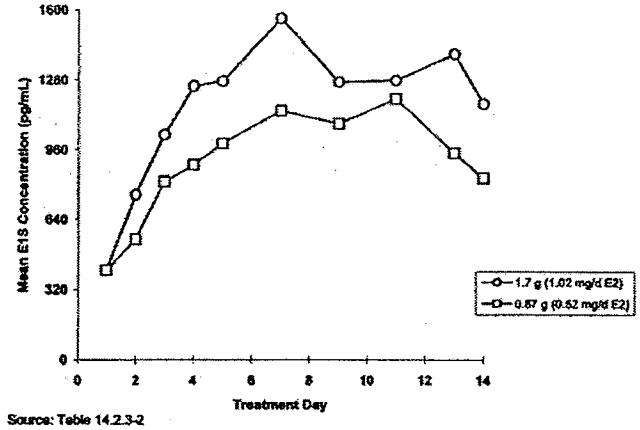
Baseline estrone levels ranged from 2.66 pg/mL to 40.33 pg/mL. after multiple application of Bio-E-Gel, the baseline adjusted exposure to E1 was approximately 5 fold higher on Day 14 than on Day 1 for both doses.

Figure 17: Mean unadjusted serum E1-S concentrations after a single dose of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 1).



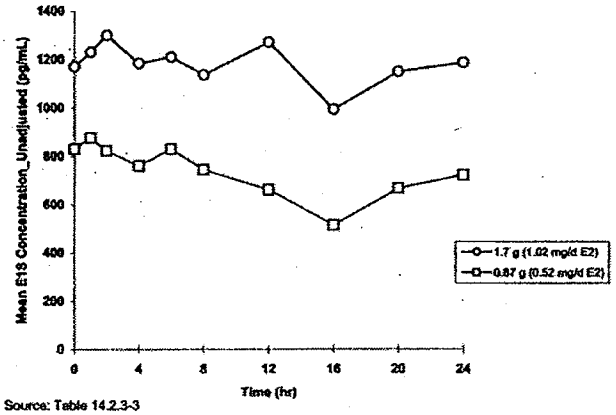
Source: Table 14.2.3-1

Figure 18: Mean unadjusted trough serum E1-S concentrations of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 1-14).



Source: Table 14.2.3-2

Figure 19: Mean unadjusted serum E1-S concentrations after multiple doses of 0.87 g (n=11) and 1.7 g (n=10) Bio-E-Gel (Day 14).



Source: Table 14.2.3-3

Table 21: Estrone Sulfate PK parameters for unadjusted serum concentrations [single dose (Day 1) and multiple dose (Day 14)]

Concentrations [Single Dose (Day 1) and Multiple Doses (Day 14)]

Treatment Group	Descriptive Statistics	$AUC_{0\text{-}24,ss}$ (pg-h/mL)	$C_{max,ss}$ (pg/mL)	$C_{ave,ss}$ (pg/mL)	$T_{max,ss}$ (h)[b]
Single Dose PK Parameters (Day 1)					
1.7 g (1.02 mg/d E2)	Mean	12529.5	762.0	522.1	24.0
	SD	6791.1	510.2	283.0	6-24
	%CV	54.2	67.0	54.2	32.5
	GeoMean	11094.6	639.0	462.3	18.7
	N	10	10	10	10
0.87 g (0.52 mg/d E2)	Mean	10354.1	567.3	431.4	24.0
	SD	4040.6	263.4	168.4	1-24
	%CV	39.0	46.4	39.0	47.9
	GeoMean	9609.9	518.2	400.4	14.3
	N	11	11	11	11
Statistical comparison for between group difference[a]		0.03	0.13	0.03	NA
Multiple Dose, Steady-state PK Parameters (Day 14)					
1.7 g (1.02 mg/d E2)	Mean	27998.5	1658.0	1166.6	4.0
	SD	17303.2	1024.7	721.0	0-24
	%CV	61.8	61.8	61.8	112.9
	GeoMean	24002.1	1406.8	1000.1	-
	N	10	10	10	10
0.87 g (0.52 mg/d E2)	Mean	16769.1	1008.2	698.7	2.0
	SD	10944.5	695.5	456.0	0-6
	%CV	65.3	69.0	65.3	91.8
	GeoMean	14766.6	872.0	615.3	-
	N	11	11	11	11
Statistical comparison for between group difference[a]		0.57	0.55	0.57	NA

[a] P-values from 2-sided t-test for between group differences. Dose-normalized values were used in statistical comparisons for dose-dependent parameters (AUC, C_{max}).
[b] For $T_{max,ss}$ median values (instead of means) and range (instead of SD) are indicated
Source: Appendices 16.1.9.2, 16.4.3.11, 16.4.3.12, 16.4.3.13 and 16.4.3.14

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Table 22: Estrone Sulfate PK parameters for adjusted serum concentrations [single dose (Day 1) and multiple dose (Day 14)]

Doses (Day14)]

Treatment Group	Descriptive Statistics	δAUC_{0-24} (pg·h/mL)	δC_{max} (pg/mL)	δC_{ave} (pg/mL)
Single Dose PK Parameters (Day 1)				
1.7 g (1.02 mg/d E2)	Mean	2982.2	354.7	124.3
	SD	2680.4	326.0	111.7
	%CV	89.9	91.9	89.9
	GeoMean	1925.7	255.4	80.2
	N	10	10	10
0.87 g (0.52 mg/d E2)	Mean	599.7	118.8	30.5
	SD	766.6	148.1	32.9
	%CV	127.8	124.6	107.8
	GeoMean	-	-	14.5
	N	11	11	9[b]
Statistical comparison for between group difference[a]		0.07	0.38	0.15
Multiple Dose, Steady-state PK Parameters (Day 14)				
1.7 g (1.02 mg/d E2)	Mean	18222.5	1250.7	759.3
	SD	12731.3	843.5	530.5
	%CV	69.9	67.4	69.9
	GeoMean	14932.7	1022.9	622.2
	N	10	10	10
0.87 g (0.52 mg/d E2)	Mean	6223.3	551.5	259.7
	SD	9775.9	651.4	407.1
	%CV	157.1	118.1	156.8
	GeoMean	2741.1	372.2	121.6
	N	11	11	11
Statistical comparison for between group difference[a]		0.41	0.73	0.41

[a] P-values from 2-sided t-test for between group differences. Dose-normalized values were used in statistical comparisons for dose-dependent parameters (AUC, C_{max}).

[b] Data from Subjects 211and 212 were set to missing.

Source: Appendices 16.1.9.2, 16.4.3.11, 16.4.3.12, 16.4.3.15 and 16.4.3.16

Study EST003 review

This was an open-labeled, multiple-dose, randomized parallel design, phase 2 study of a topical administration of Bio-E-Gel in twelve postmenopausal women.

The primary objectives were to evaluate the safety, tolerability, and pharmacokinetic profile of two different, multiple topical doses of Bio-E-Gel (1.25 and 2.5 g/day) in terms of the PK variables AUC and C_{max} with and without corrections for endogenous estradiol concentrations.

12 subjects were randomly receive either Bio-E-Gel 1.25 (n=6) or 2.5 (n=6) g once daily for 14 days applied to the front and inner area of one thigh. Trough levels were measured on Day 1 prior to first dose and on Days 2, 3, 4, 5, 7, 9, 11, 13, and 14 prior to application of the next dose. Additional samplings occurred on Days 16, 18, and 20 at same trough time as on Day 15. Two samples (at -16 and -10 hour) were taken on Day 0, and together with trough level on Day 1, their mean makes up the baseline level. This single baseline value from each subject was subtracted from all observed concentrations from that subject for 'net absorption' calculations. Negative net absorption concentrations were set to zero.

Intensive PK samplings were done on Day 1 (at 1, 2, 4, 6, 8, 12, 16, and 24 hours postdose) and Day 14 (at 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours postdose).

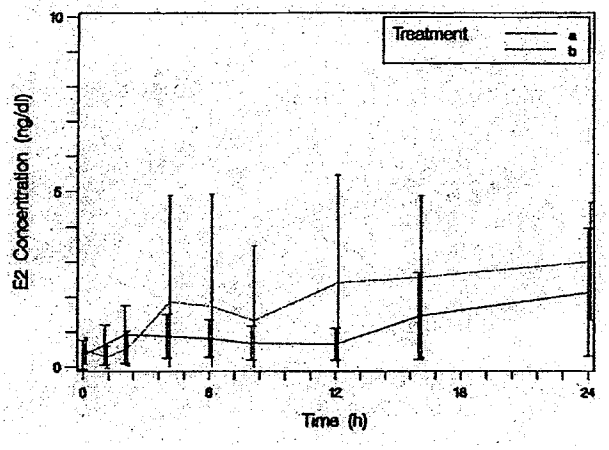
Estradiol, estrone, estrone sulfate, and sex hormone binding globulin (SHBG) levels were measured using validated assays.

Results:

Estradiol:

Following single dose of Bio-E-Gel, on average E2 concentration increased from a baseline of 0.4 ng/dl at 0h to 2.1 ng/dl at 24 h for the 1.25 g dose. For the 2.5 g dose, E2 concentration increased from 0.5 ng/dl at 0 h to 3.0 ng/dl at 24 h. The higher dose resulted in higher exposure. The figure below shows the mean serum concentration following single dose administration.

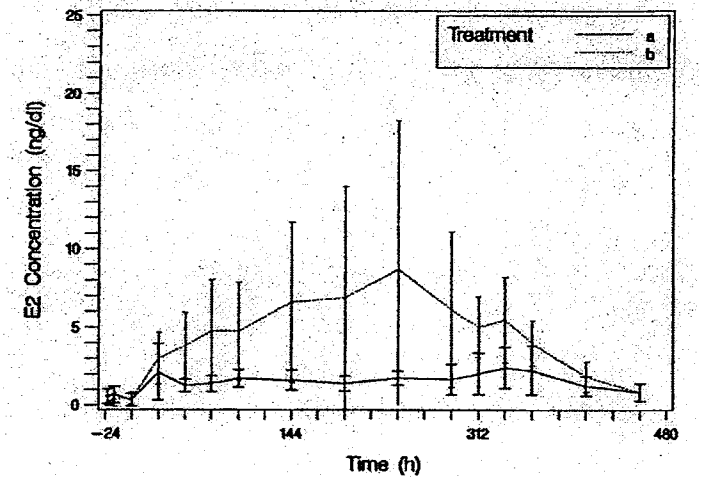
Figure 20: Mean unadjusted E2 serum concentrations following single dose of 1.25 g (a) or 2.5 g (b) Bio-E-Gel. Error bars represent standard deviations (n=6).



The figure below shows the trough concentrations bor both 1.25 and 2.5 g doses. The mean E2 concentration reach a plateau quickly after about 2 days for the 1.25 g dose but continued to rise until day 11 (predose) for the 2.5 g dose. The continued rise of the 2.5 g dose was driven by an apparent outlier that had very high level with a peak of 28 ng/dl on day 11 (predose). Median E2

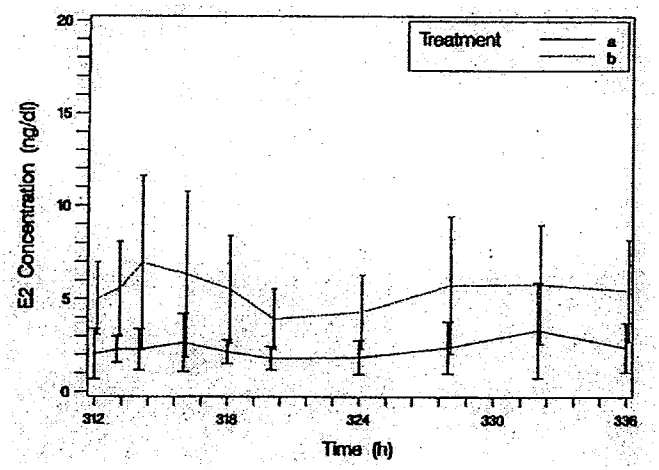
values were used to estimate that steady state was reached by 4 and 5 days for the Bio-E-Gel 1.25 and 2.5 g, respectively.

Figure 21: Mean unadjusted trough serum E2 concentrations of 1.25 g (a) and 2.5 g (b) Bio-E-Gel (Day 1-14). Error bars represent standard deviations (n=6).



E2 levels on Day 14 shows an apparent plateau with some intra-day variation (see figure below). The lack of rise as observed on Day 1 suggests steady state has been reached by Day 14.

Figure 22: Mean unadjusted serum E2 concentrations after multiple doses of 1.25 g (a) and 2.5 g (b) Bio-E-Gel (Day 14). Error bars represent standard deviations (n=6).



The PK parameters for single and multiple doses Bio-E-Gel are presented in table below. Exposure on Day 14 was higher than that of Day 1, indicating accumulation of E2 in the serum following repeated application. Ratios of mean E2 on Day 14/Day 1 are 2.07 and 2.58 for 1.25 g and 2.5 dose, respectively.

Table 23: E2 PK parameters by dose regimens

Variable	Statistic	1.25 g Bio-E-Gel, Single Dose	1.25 g Bio-E-Gel, Multiple Dose	2.5 g Bio-E-Gel, Single Dose	2.5 g Bio-E-Gel, Multiple Dose
AUC_τ [ng/dl*H]	N	6	6	6	6
	Mean	27.5	56.8	49.7	128.2
	SD	17.2	30.2	48.1	50.0
	GeoM	19.2	51.5	38.4	117.6
	G_CV	173.5	49.4	79.0	53.1
C_{max} [ng/dl]	N	6	6	6	6
	Mean	2.3	3.6	3.7	8.8
	SD	1.8	2.4	2.7	4.8
	GeoM	1.7	3.1	3.1	7.6
	G_CV	110.1	62.0	75.9	67.0
t_{max} [H]	N	6	6	6	6
	Mean	17.67	324.00	18.00	330.33
	SD	8.62	10.49	4.90	8.62
	Min	2.00	313.00	12.00	314.00
	Med	20.00	325.00	16.00	334.00
	Max	24.00	336.00	24.00	336.00
Baseline, C_0 [ng/dl]	N	6	6	6	6
	Mean	0.5	0.5	0.4	0.4
	SD	0.4	0.4	0.3	0.3
	Min	0.0	0.0	0.0	0.0
	Med	0.5	0.5	0.4	0.4
	Max	1.3	1.3	0.8	0.8

Sponsor calculated the ratio of geometric mean of Bio-E-Gel 2.5 g/Bio-E-Gel 1.25 g to assess the dose proportionality of E2 following these 2 doses. The ratios are 2.0 and 2.3 for single and multiple doses, respectively. The sponsor proposed that this indicated dose proportionality. This reviewer believes a better method would be to use the baseline corrected AUC to estimate dose proportionality of Bio-E-Gel. Using baseline corrected AUC, the ratios are 2.57 and 2.79 for single and multiple dose, respectively, suggesting a higher than dose proportional increase in exposure.

Baseline correction. The mean baseline E2 were 0.5 ng/dl and 0.4 ng/dl for the 1.25 nd 2.5 g Bio-E-Gel, respectively. To correct for endogenous E2, the individual subject's baseline E2 concentration was subtracted from each concentration for that subject and PK parameters were calculated from the baseline-adjusted concentrations. The summary of PK parameters is listed in the table below. In general, AUC and C_{max} are greater with multiple dose suggesting drug accumulation over 14 days. Ratios of mean baseline-adjusted E2 on Day 14/Day 1 are 2.95 and 2.89 for 1.25 g and 2.5 dose, respectively.

The terminal $t_{½}$ of E2 was calculated from the baseline-adjusted concentration following the last dose on Day 14 at 312 h. The mean estimated $t_{½}$ following the application of 1.25 g Bio-E-Gel and 2.5 g Bio-E-Gel were 40.33 ± 31.59 and 37.34 ± 12.41 h, respectively. The median $t_{½}$ following the application of 1.25 g Bio-E-Gel and 2.5 g Bio-E-Gel were 24.49 and 35.58 h, respectively.

Table 24: E2 PK parameters, baseline adjusted

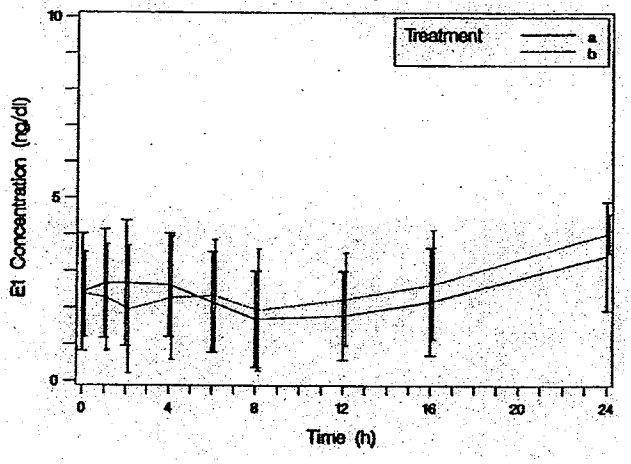
Variable	Statistic	1.25 g Bio-E-Gel, Single Dose	1.25 g Bio-E-Gel, Multiple Dose	2.5 g Bio-E-Gel, Single Dose	2.5 g Bio-E-Gel, Multiple Dose
δAUC, [ng/dl*H]	N	6	6	6	6
	Mean	14.9	44.0	41.4	119.6
	SD	13.3	22.6	51.1	51.2
	GeoM	9.8	39.1	25.2	108.9
	G_CV	147.3	59.8	139.6	53.5
δC_{max} [ng/dl]	N	6	6	6	6
	Mean	1.8	3.0	3.4	8.4
	SD	1.8	2.1	2.9	4.7
	GeoM	1.2	2.5	2.4	7.3
	G_CV	132.8	72.0	118.5	68.5
$t_{1/2}$ [H]	N		3		4
	Mean		40.33		37.34
	SD		31.59		12.41
	Min		19.80		26.60
	Med		24.49		35.58
	Max		76.71		51.59

Estrone:

In general, the PK of estrone is qualitatively similar to that of E2.

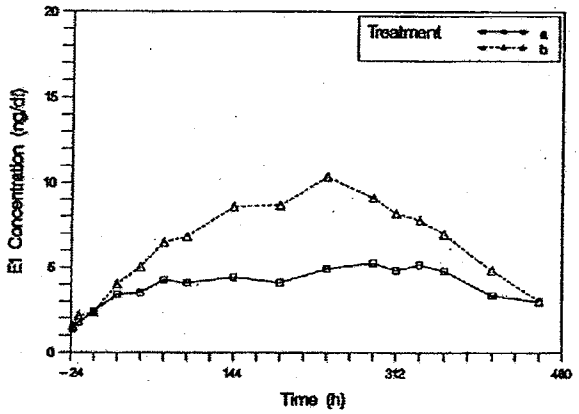
Following single dose of Bio-E-Gel, on average E1 concentration increased from a baseline of 2.4 ng/dl at 0h to 3.4 ng/dl at 24 h for the 1.25 g dose. For the 2.5 g dose, E1 concentration increased from 2.4 ng/dl at 0 h to 4.0 ng/dl at 24 h. The higher dose resulted in only slightly higher exposure after a single dose. The figure below shows the mean serum concentration following single dose administration.

Figure 23: Mean unadjusted E1 serum concentrations following single dose of 1.25 g (a) or 2.5 g (b) Bio-E-Gel. Error bars represent standard deviations (n=6).



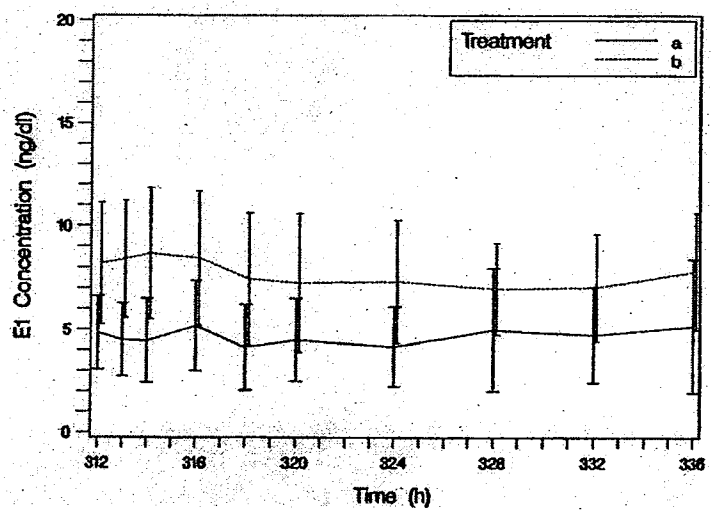
The figure below shows the trough concentrations for both 1.25 and 2.5 g doses. The mean E1 concentration reach a plateau quickly after about 4 days for the 1.25 g dose but continued to rise until day 11 (predose) for the 2.5 g dose. Examination of mean E1 trough values indicates that steady state concentrations are reached by 11 and 13 days for the Bio-E-Gel 1.25 and 2.5 g, respectively.

Figure 24: Mean unadjusted trough serum E1 concentrations of 1.25 g (a) and 2.5 g (b) Bio-E-Gel (Day 1-14). Error bars represent standard deviations (n=6).



E1 levels on Day 14 shows an apparent plateau with little intra-day variation (see figure below). The lack of rise as observed on Day 1 suggests steady state has been reached by Day 14.

Figure 25: Mean unadjusted serum E1 concentrations after multiple doses of 1.25 g (a) and 2.5 g (b) Bio-E-Gel (Day 14). Error bars represent standard deviations (n=6).



The E1 PK parameters for single and multiple doses Bio-E-Gel are presented in table below. Exposure on Day 14 was higher than that of Day 1, indicating accumulation of E1 in the serum following repeated application. Ratios of mean E1 on Day 14/Day 1 are 1.98 and 2.89 for 1.25 g and 2.5 dose, respectively.

Table 25: E1 PK parameters by dose regimens, unadjusted

Variable	Statistic	1.25 g Bio-E-Gel, Single Dose	1.25 g Bio-E-Gel, Multiple Dose	2.5 g Bio-E-Gel, Single Dose	2.5 g Bio-E-Gel, Multiple Dose
AUC, [ng/dl*H]	N	6	6	6	6
	Mean	56.2	111.4	62.2	179.7
	SD	31.2	54.2	30.0	67.6
	GeoM	49.6	100.8	56.2	167.1
	G_CV	59.2	51.9	53.2	46.3
C_{max} [ng/dl]	N	6	6	6	6
	Mean	3.6	6.0	4.1	9.2
	SD	1.6	2.7	0.6	3.1
	GeoM	3.2	5.6	4.0	8.7
	G_CV	56.3	45.0	13.2	40.5
t_{max} [H]	N	6	6	6	6
	Mean	12.67	323.33	21.01	314.33
	SD	12.42	9.93	7.33	1.51
	Min	1.00	312.00	6.05	312.00
	Med	13.00	322.00	24.00	314.00
	Max	24.00	336.00	24.00	316.00
Baseline, C_0 [ng/dl]	N	6	6	6	6
	Mean	1.8	1.8	2.0	2.0
	SD	1.4	1.4	0.9	0.9
	Min	0.5	0.5	1.1	1.1
	Med	1.5	1.5	1.8	1.8
	Max	4.4	4.4	3.2	3.2

Baseline correction. The mean baseline E1 were 1.8 ng/dl and 2.0 ng/dl for the 1.25 nd 2.5 g Bio-E-Gel, respectively. To correct for endogenous E1, the individual subject's baseline E1 concentration was subtracted from each concentration for that subject and PK parameters were calculated from the baseline-adjusted concentrations. The summary of PK parameters is listed in the table below. In general, AUC and C_{max} are greater with multiple dose suggesting drug accumulation over 14 days. Ratios of mean baseline-adjusted E1 on Day 14/Day 1 are 4.63 and 7.33 for 1.25 g and 2.5 dose, respectively.

Table 26: E1 PK parameters, baseline adjusted

Table 11.5.3 d: E1 – PK Variables, Baseline Adjusted

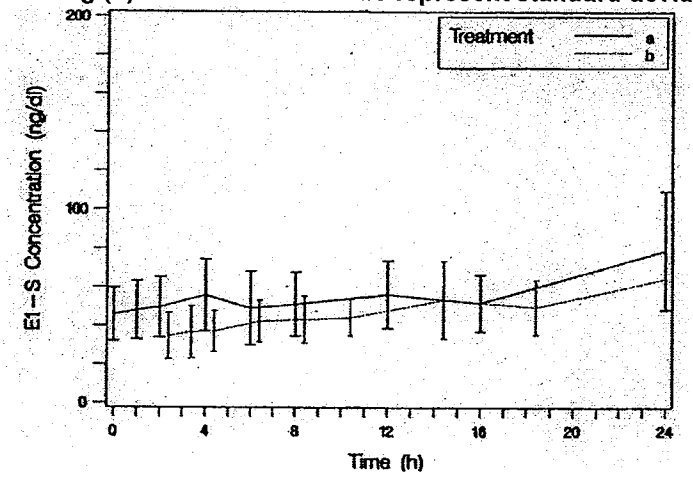
Variable	Statistic	1.25 g Bio-E-Gel, Single Dose	1.25 g Bio-E-Gel, Multiple Dose	2.5 g Bio-E-Gel, Single Dose	2.5 g Bio-E-Gel, Multiple Dose
δAUC_τ [ng/dl*H]	N	6	6	6	6
	Mean	14.5	67.1	17.9	131.2
	SD	5.6	27.1	6.0	68.4
	Med	14.6	63.9	16.2	139.6
	GeoM	13.6	63.0	17.2	113.8
	G_CV	42.1	39.9	31.3	68.0
δC_{max} [ng/dl]	N	6	6	6	6
	Mean	1.8	4.2	2.0	7.2
	SD	0.8	1.7	0.5	3.2
	Med	1.7	3.6	2.0	8.0
	GeoM	1.6	4.0	2.0	6.4
	G_CV	47.2	34.7	30.6	57.5

Estrone sulfate:

Following single dose of Bio-E-Gel, on average E1-S concentration increased from a baseline of 45.8 ng/dl at 0h to 79.0 ng/dl at 24 h for the 1.25 g dose. For the 2.5 g dose, E1-S concentration increased from 34.7 ng/dl at 0 h to 70.7 ng/dl at 24 h. The higher dose actually resulted in slightly lower exposure after a single dose due to lower baseline at 0 h. The concentration difference

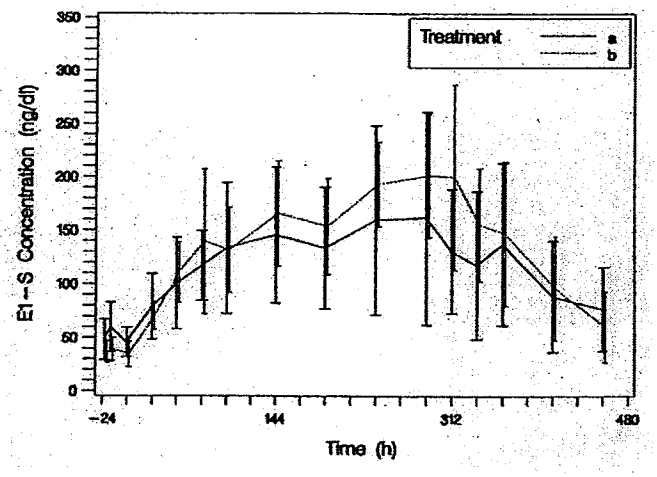
between 24 h and 0 h was slightly higher for the 2.5 g dose. The figure below shows the mean serum concentration following single dose administration.

Figure 26: Mean unadjusted E1-S serum concentrations following single dose of 1.25 g (a) or 2.5 g (b) Bio-E-Gel. Error bars represent standard deviations (n=6).



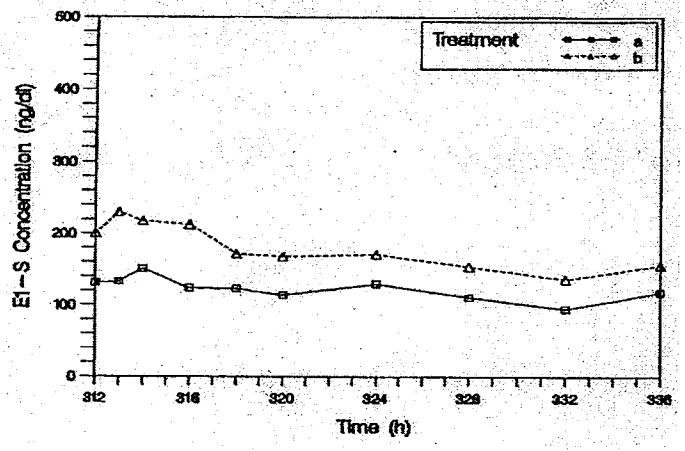
The figure below shows the trough concentrations for both 1.25 and 2.5 g doses. The mean trough concentration appears to increase with repeated administration for the entire study duration. The rate of increase appeared to change at Day 9 predose for 1.25 g dose and Day 11 predose for 2.5 g dose. The sponsor's examination of mean trough indicates that steady state E1-S concentration are reached by 13 and 14 days for the bio-e-gel 1.25 and 2.5 g doses, respectively. Five days following the last dose, E1-S levels were still higher than baseline for both dose groups.

Figure 27: Mean unadjusted trough serum E1-S concentrations of 1.25 g (a) and 2.5 g (b) Bio-E-Gel (Day 1-14). Error bars represent standard deviations (n=6).



E1-S levels on Day 14 show an apparent decrease over the 24 hour period (see figure below).

Figure 28: Mean unadjusted serum E1-S concentrations after multiple doses of 1.25 g (a) and 2.5 g (b) Bio-E-Gel (Day 14). Error bars represent standard deviations (n=6).



The PK parameters for single and multiple doses Bio-E-Gel are presented in table below. Exposure on Day 14 was higher than that of Day 1, indicating accumulation of E1-S in the serum following repeated application. Ratios of mean E1-S on Day 14/Day 1 are 2.09 and 3.38 for 1.25 g and 2.5 dose, respectively.

Table 27: E1-sulfate PK parameters by dose regimens, unadjusted

Variable	Statistic	1.25 g Bio-E-Gel, Single Dose	1.25 g Bio-E-Gel, Multiple Dose	2.5 g Bio-E-Gel, Single Dose	2.5 g Bio-E-Gel, Multiple Dose
AUC_t [ng/dl*H]	N	6	6	6	6
	Mean	1359.2	2834.1	1207.4	4079.2
	SD	407.8	1219.0	243.6	1674.5
	GeoM	1302.6	2611.1	1184.3	3798.7
	G_CV	33.9	47.2	22.6	43.4
C_{max} [ng/dl]	N	6	6	6	6
	Mean	80.2	163.5	74.7	253.8
	SD	30.5	75.5	12.1	124.2
	GeoM	75.2	148.2	73.8	231.3
	G_CV	41.5	52.6	17.0	49.0
t_{max} [H]	N	6	6	6	6
	Mean	20.67	316.67	20.00	315.33
	SD	8.16	3.93	6.20	4.46
	Min	4.00	314.00	12.00	312.00
	Med	24.00	315.00	24.00	313.50
	Max	24.00	324.00	24.00	324.00
Baseline, C_0 [ng/dl]	N	6	6	6	6
	Mean	51.3	51.3	36.9	36.9
	SD	17.9	17.9	10.7	10.7
	Min	23.3	23.3	23.3	23.3
	Med	55.5	55.5	38.0	38.0
	Max	71.0	71.0	53.0	53.0

Baseline correction. The mean baseline E1-S were 51.3 ng/dl and 36.9 ng/dl for the 1.25 nd 2.5 g Bio-E-Gel, respectively. To correct for endogenous E1-S, the individual subject's baseline E1-S concentration was subtracted from each concentration for that subject and PK parameters were calculated from the baseline-adjusted concentrations. The summary of PK parameters is listed in the table below. In general, AUC and C_{max} are greater with multiple dose suggesting drug accumulation over 14 days. Ratios of mean baseline-adjusted E1-S on Day 14/Day 1 are 9.65 and 9.79 for 1.25 g and 2.5 dose, respectively. The baseline-adjusted accumulation for E1-S was > E1 > E2.

Table 28: E1-sulfate PK parameters, baseline adjusted

Variable	Statistic	1.25 g Bio-E-Gel, Single Dose	1.25 g Bio-E-Gel, Multiple Dose	2.5 g Bio-E-Gel, Single Dose	2.5 g Bio-E-Gel, Multiple Dose
δAUC_t [ng/dl*H]	N	6	6	6	6
	Mean	165.7	1602.1	325.5	3192.5
	SD	63.7	878.6	267.1	1543.4
	GeoM	153.9	1403.2	256.1	2893.6
	G_CV	46.4	61.9	87.6	51.8
δC_{max} [ng/dl]	N	6	6	6	6
	Mean	28.8	112.2	37.7	216.9
	SD	19.3	61.0	16.0	120.4
	GeoM	24.1	97.0	33.7	192.9
	G_CV	71.7	67.2	63.6	55.7

Sex hormone binding globulin (SHBG):

SHBG were measured to assess the effect of Bio-E-Gel administration on SHBG levels and to help explain the very high E2 level in subject 4. SHBG level increased from 72.5 nM at baseline to 80.17 nM at 192 h and 84.00 nM at 360 h after first application of 1.25 g/day Bio-E-Gel. For the 2.5 g dose, the level increased from 72.5 nM, to 77.83, and to 88.83 nM at baseline, 192, and 360 h, respectively. A summary of finding is listed in the table below. Subject 4 high E2 levels could not be explained by high SHBG level as he/she did not have abnormal SHBG.

Table 29: SHBG [nM]

Treatment	Statistic	Scheduled time relative to first application			
		-16	-10	192	360
Bio-E-Gel, 1.25 g	N	6	6	6	6
	Mean	72.33	72.50	80.17	84.00
	SD	23.73	24.83	28.53	29.18
	GeoM	69.12	69.02	75.94	79.73
	G_CV	34.09	35.54	37.59	36.85
Bio-E-Gel, 2.5 g	N	6	6	6	6
	Mean	74.00	72.50	77.83	88.83
	SD	29.64	30.34	32.17	38.97
	GeoM	68.91	67.20	72.20	81.17
	G_CV	43.88	45.10	45.01	50.71

Study EST006 review

Study EST006 was a Phase 1, parallel group, randomized, open label clinical study. The objective of this study was to evaluate the potential transfer of estradiol (E2) from female subjects dosed with 2.6 g Bio-E-Gel (1.56 mg E2) to untreated male subjects through skin-to-skin contact. The amount of E2 remaining on the skin of females and potentially transferable was also evaluated at 2 and 8 hours following bio-e-gel application and then after washing the dosed area 8 hours post Bio-E-Gel application.

This study included 24 female/male couples (24 postmenopausal females and 24 males). Twelve couples were assigned to treatment group 1 and 12 to treatment group 2.

To establish baseline E2 levels, males in both group 1 and 2 (n=24) underwent serum E2 sampling on Day -1 at 0 h (pre-skin-to-skin contact time specified for Day 1), and at 1, 2, 4, 8 and 24 hours relative to the projected skin-to-skin contact specified on Day 1. On Day 1, Bio-E-Gel was applied topically to each arm of females in group 1, and to a single arm in the group 2 females.

Two hours after dosing, Group 1 females underwent application site swabbing on one arm, followed by using the opposite arm to engage in vigorous arm-to-arm contact with a male subject for 5 continuous minutes. Serum E2 sampling from the male's opposite arm (to reduce risk of contamination) occurred just prior to skin rubbing (0 h, Day 1) and at 1, 2, 4, 8, 24, and 48 hours after skin rubbing. The group 1 females again underwent application site swabbing 8 hours after dosing, followed by washing of the arm, and additional application site swabbing. At each designated swab time, a surface area of 20 cm^2 ($1/16^{th}$ of the total application site) was swabbed with 3 cotton tipped swabs.

Group 2 couples under went the same arm-to-arm contact procedures except that it commenced 8 hours (instead of 2 hours) after the Bio-E-Gel application and E2 blood sampling occurred from the opposite arm of the males just as in group 1. No application site swabbing occurred in the group 2 females.

Table 30: study design

Study group	Number of subjects (male/female)	Treatment drug	Dose	Dosing regimen
1	12/12	Bio-E-Gel (0.06% E2 gel)	5.2 g gel (1.56 mg E2) given as 2 x 2.6 g	Two topical applications; one to each upper arm
2	12/12	Bio-E-Gel (0.06% E2 gel)	2.6 g gel (1.56 mg E2)	One topical application to upper arm

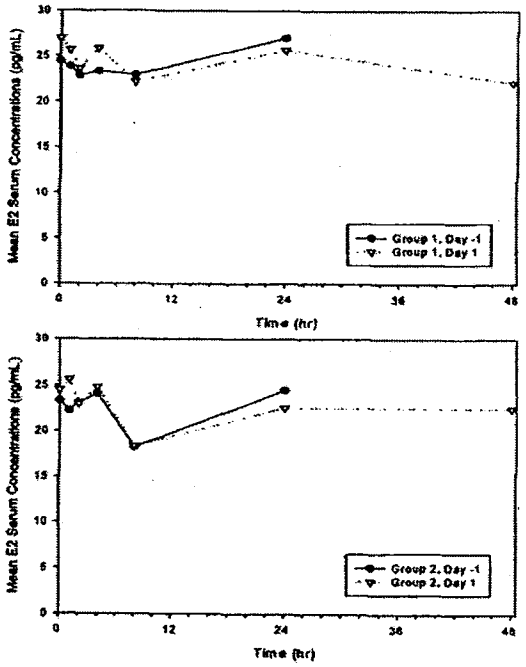
Serum E2 concentration in males were measured using a validated radioimmunoassay method. The lower limit of quantitation for E2 was 0.5 ng/dL (5 pg/mL) using a 2 mL sample. E2 concentration in the swabs was measured using a LC/MS/MS assay with 2 ranges, namely 0.5 to 500 ng/mL and 400 to 200,000 ng/mL. All swab samples were assayed with the 400 to 200,000 ng/mL standard curve first and samples that fell below LOQ were reanalyzed using the lower range curve. The concentration assayed range from 49 ng/mL to 4370 ng/mL. Recovery was estimated to be 92%, but it was not clear if this was accounted for in the reported concentration.

PK analysis:
To assess the potential for E2 transfer, the primary endpoints were AUC_{0-24}, and C_{max}. Baseline was adjusted using time 0 h concentration value of there respective day. E2 24 hour profile during Day -1 was used to compare the diurnal patterns prior to and subsequent to E2 transfer.

To assess the effect of application site washing, the primary endpoint is the residual E2 at 2 and 8 hours post application and after washing the application area at 8 hours post Bio-E-Gel application.

Results:
For each group, Day 1 E2 concentrations were similar and followed the same pattern as on Day -1, indicating no change in a subject's normal circadian E2 pattern as a result of skin-to-skin contact. Additionally, the E2 level decreased overtime after skin-to-skin contact, suggesting that there was no significant absorption of E2 following a single incident of skin-to-skin drug contact.

Figure 29: Male partner unadjusted E2 mean serum concentration by treatment day



Group 1: Skin-to-Skin contact at 2 h
Group 2: Skin-to-Skin contact at 8 h

Day 1 E2 concentrations were similar between both groups and followed a similar circadian pattern as Day -1. Exposure of E2 (described as AUC_{0-24}, C_{max} and C_{ave}) was not significantly different between Day -1 and Day 1, nor did exposure differ between Group 1 and Group 2. No differences in exposures between groups were observed when Day -1 and Day 1 parameters were calculated based on adjustment of concentrations for baseline.

Table 31: Unadjusted E2 serum PK parameters in untreated males before and after ski-to-skin contact.

Treatment Day	Descriptive Statistics	$AUC_{0\text{-}24}$ (pg*h/mL)		$C_{max\,0\text{-}24}$ (pg/mL)		$C_{ave\,0\text{-}24}$ (pg/mL)		$T_{max,0\text{-}24}$ (h)[b]	
		Group 1 (2h)	Group 2 (8h)	Group 1 (2h)	Group 2 (8h)	Group 1 (2h)	Group 2 (8h)	Group 1 (2h)	Group 2 (8h)
Day -1	Mean	585.5	519.3	29.8	28.8	24.4	21.6	1.0	4.0
	SD	212.3	150.6	9.5	10.2	8.8	6.3	0 - 24	0 - 24
	%CV	36.3	29.0	31.8	35.5	36.3	29.0	127.3	114.3
	GeoMean	547.0	502.4	28.3	27.4	22.8	20.9	-	-
	N	12	12	12	12	12	12	12	12
Day 1	Mean	579.0	519.3	31.2	29.3	24.1	21.2	2.5	1.5
	SD	161.5	159.1	10.1	11.5	6.7	5.9	0 - 24	0 - 24
	%CV	27.9	30.6	32.5	39.4	27.9	27.6	134.1	175.8
	GeoMean	557.2	499.2	29.6	27.4	23.2	20.5	-	-
	N	12	12	12	12	12	12	12	12
Statistical comparison for within group difference[a]		0.93	0.9997	0.74	0.91	NA	NA	NA	NA

[a] p-values from 2-sided t-test for within group difference (Day -1, 0-24 h vs Day 1, 0-24 h).
[b] For T_{max}, median values (instead of means) and range (instead of SD) are indicated.

Table 32: Baseline adjusted E2 serum PK parameters in untreated males before and after skin-to-skin contact. Baseline values were individual E2 concentration at 0h on the respective Day.

Treatment Day	Descriptive Statistics	$\delta AUC_{0\text{-}24}$ (pg*h/mL)		δC_{max} (pg/mL)		δC_{ave} (pg/mL)	
		Group 1 (2h)	Group 2 (8h)	Group 1 (2h)	Group 2 (8h)	Group 1 (2h)	Group 2 (8h)
Day -1	Mean	-0.5	-40.7	5.4	5.4	-0.0	-1.7
	SD	130.7	99.2	5.6	2.6	5.4	4.1
	%CV	-24131.7	-243.6	103.7	48.8	-24131.7	-243.6
	N	12	12	12	12	12	12
Day 1	Mean	-69.0	-78.6	4.2	4.8	-2.9	-3.3
	SD	162.6	182.8	7.3	5.3	6.8	7.6
	%CV	-235.7	-232.5	174.2	111.1	-235.7	-232.5
	N	12	12	12	12	12	12
Statistical comparison for within group difference[a]		0.32	0.34	0.54	0.62	0.32	0.34

[a] p-values from 2-sided t-test for within group difference (Day -1, 0-24 h vs Day 1, 0-24 h).

The mean percent of E2 recovered from the skin at 2 and 8 h post-application was 4.6 ± 4.0 % and 7.8 ± 5.8 % of the applied dose of E2, respectively. Washing the application area with soap and water 8 h post-application decreased the percent of E2 recovered from the skin to [redacted]% of the applied dose. This suggests that washing of the application site area substantially decreased the potential for transfer of Bio-E-Gel.

Table 33: Estimated % of applied E2 recovered from skin. Corrections for surface area swabbed were done. The procedure assumes that the swabbing remove all estradiol available at the surface of skin.

Statistic	Day 1		
	2 h Post Application	8 h Post Application	8 h Post Application and Washing
Mean	4.58[a, c]	7.76[a, b]	1.38[b, c]
SD	3.96	5.79	1.06
%CV	86.35	74.61	76.69
2-sided t-test comparison, p-value	a	b	c
	0.131	0.001	0.013

Note: Amount of E2 recovered from 20 cm^2 was multiplied by 16 to give the amount recoverable from the entire application area of 320 cm^2.
Source: Appendix 16.4.4.3

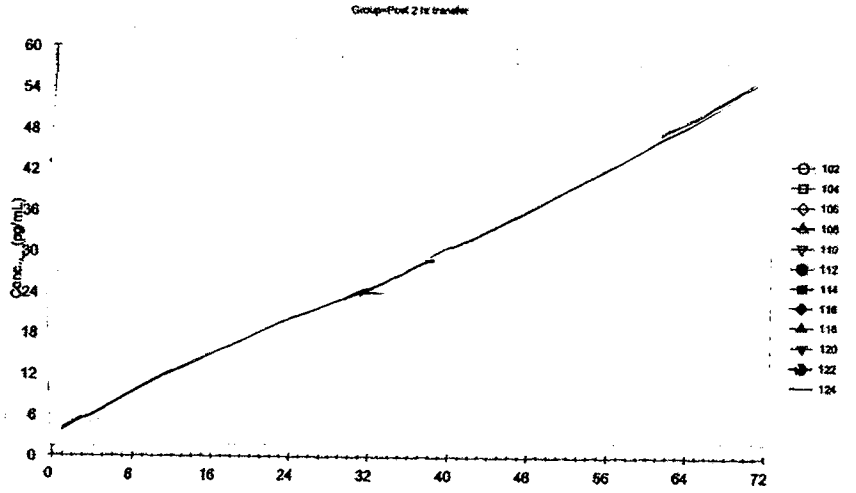
Conclusions:
Following physical contact (vigorous skin-to-skin rubbing) for 5 min at 2 and 8 h post application between dosed female subjects and untreated males, mean E2 serum concentrations were similar for both groups. For both contact groups, exposures of E2 (described as δAUC0-24 and δCave) decreased or only slightly increased (δCmax) from baseline values, indicating no accumulation of drug as a result of a single incident of skin-to-skin contact. Day 1 concentrations were similar and followed a similar circadian pattern as Day -1. Exposures of E2 (described as $AUC_{0\text{-}24}$, C_{max} and C_{ave}) were not significantly different between Day -1 and Day 1, nor did they appear to be different between Group 1 and Group 2. No differences in exposures between groups were observed when Day -1 and Day 1 parameters were calculated based on adjustment of concentrations for baseline.

The mean percent of E2 recovered from the skin at 2 and 8 h post-application was 4.6 ± 4.0 % and 7.8 ± 5.8 % of the applied dose of E2, respectively. Washing the application area with soap and water 8 h post-application decreased the percent of E2 recovered from the skin to [redacted]% of the applied dose. This suggests that washing of the application site area substantially decreased the potential for transfer of Bio-E-Gel. It is not clear if 92% assay extraction recovery was used in calculation of skin recovery. However, this is minor considering the low residual estradiol level on the skin at 2 and 8 hours post dose.

No clinically important adverse events, including skin irritation, were observed during the trial. No subject died or experienced a serious adverse event, and no subject was withdrawn from the study because of an adverse event.

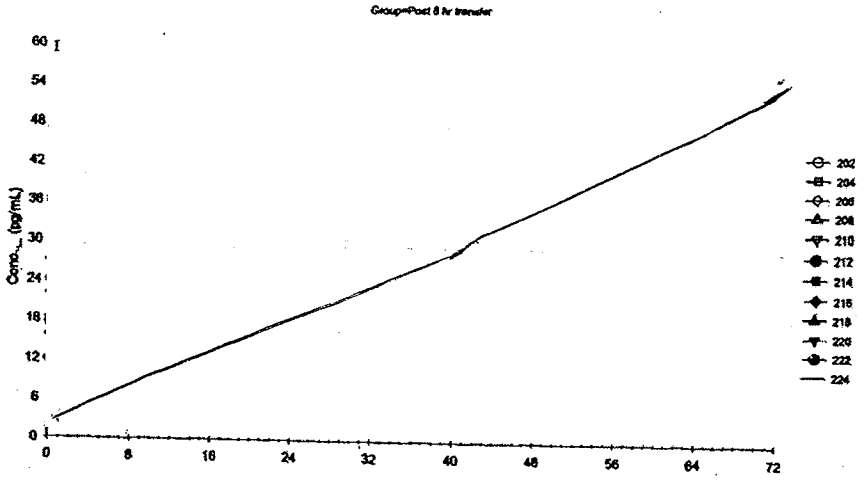
The next 3 figures show the raw data for all subjects.

Figure 30: Group 1 male E2 individual serum concentrations at baseline (Day -1, 0-24h) and after skin-to-skin contact at 2 h post dose (Day 1 to 3, 24-72 h)



Source: Appendix 16.4.3.1 **Time Day -1 to Day 3 (hr)**

Figure 31 Group 2 male E2 individual serum concentrations at baseline (Day -1, 0-24h) and after skin-to-skin contact at 8 h post dose (Day 1 to 3, 24-72 h)



Source: Appendix 16.4.3.2 **Time Day -1 to Day 3 (hr)**

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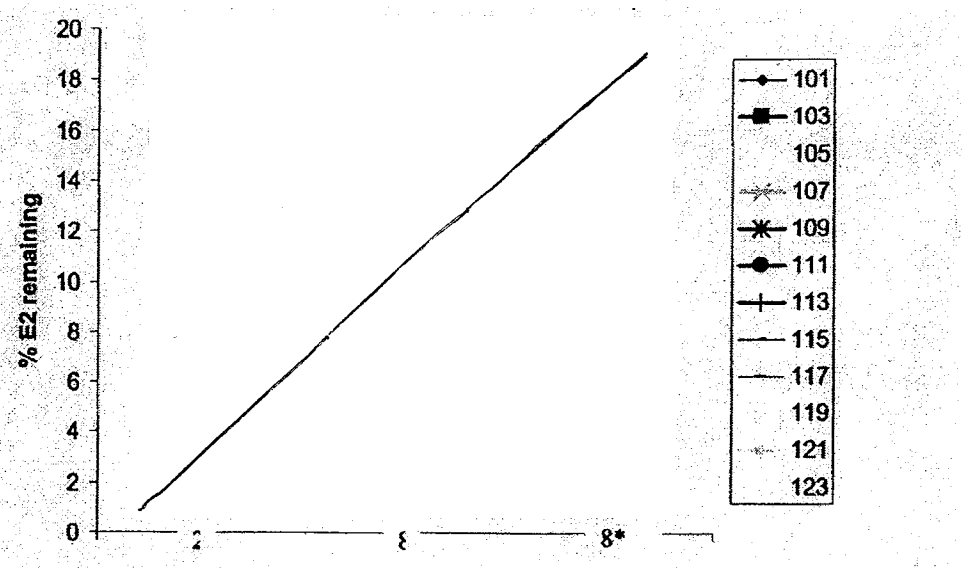
Figure 32: Group 1 dosed females. Percent of E2 remaining on the skin (2 h and 8 h) and post application site washing (8* h).



* - Post application site washing
Source: Appendices 16.4.4.3 and 16.4.4

Study EST008 Review

This was a Phase 1, single-center, randomized, open-label, two-period crossover, multiple-dose study to evaluate the effect of administering sunscreen and Bio-E-Gel on hormone levels in post menopausal women.

The objective of this study was to evaluate serum hormone levels and pharmacokinetic (PK) parameters for estradiol (E2), estrone (E1) and estrone sulfate (E1S) in postmenopausal women following multiple applications of Bio-E-Gel 2.6 g (1.56 mg E2), when a sunscreen product ([ ] [ ] (SPF 30 UV A,UVB) sunscreen lotion) was applied 10 min before or 25 min after a once daily application of Bio-E-Gel.

Design:
This was a single-center, open-label, randomized, multiple-dose, 2-period crossover, PK study in 12 postmenopausal females. After meeting the medical history, physical examination and laboratory testing qualifications at screening, the women were enrolled in the study and randomized into 2 groups; each comprised 6 subjects.

Each group received once daily application of 2.6 g of Bio-E-Gel for 15 days to achieve steady-state serum hormone levels. Blood draws were performed pre-dose on Days 13-14 to determine trough steady-state serum hormone levels ($C_{min, ss}$ or $trough_{ss}$). Serial blood draws were taken throughout a 24 h period on Day 15 for serum hormone analyses, which were used to determine $AUC_{0-24, ss}$.

One group (Sequence 1) then applied sunscreen 10 min before each application of Bio-E-Gel for the next 7 days (Days 16-22). Serial blood draws were taken pre-dose and throughout a 24 h period beginning on Day 22 for serum hormone levels, which were used to determine $AUC_{0-24, ss}$ and $trough_{ss}$. Following another 15 day period (Days 23-37) of receiving a once daily application of 2.6 g Bio-E-Gel, blood draws were performed pre-dose on Days 35-36 to determine $trough_{ss}$. Serial blood draws were taken throughout a 24 h period beginning on Day 37 for serum hormone analyses, which were used to determine $AUC_{0-24,ss}$ and $trough_{ss}$. Subsequently, these subjects applied sunscreen 25 min after the application ofBio-E-Gel for the final 7 days of the study (Days 38-44). On the day of the final application ofBio-E-Gel and sunscreen, serial blood draws were taken pre-dose and throughout a 24 h period beginning on Day 44 for serum hormone levels, which were used to determine $AUC_{0-24, ss}$ and $trough_{ss}$.

The second group (Sequence 2) of 6 subjects received the same therapies, but received the sunscreen applications in the opposite sequence, i.e. 25 min after Bio-E-Gel for the first 7 days (Days 16-22), and 10 min before Bio-E-Gel application for the last 7 days (Days 38-44) of Bio-E-Gel and sunscreen combined therapy.

All subjects received a brief physical examination and provided blood or urine for laboratory testing (hematology, chemistry and urinalysis) at screening and end of study (EOS; Day 45) to monitor safety. Subjects were queried for treatment-emergent adverse events (AEs) prior to each application of Bio-E-Gel and for 6 days after the last application of the gel (Day 50).

Analytical Assay:
Serum concentrations of E2, E1 and EIS were determined using validated radioimunoassay (RIA) methods. The lower limit of quantitation (LLOQ) was 0.5 ng/dL (5 pg/mL) for E2, 0.5 ng/dL (5 pg/mL) for E1, and 10 ng/dL (100 pg/mL) for E1S.

Pharmacokinetic Evaluation:
The PK parameters of AUC_{0-24}, C_{max}, and $_{Cave}$, were calculated from steady-state, serum E2, E1, and E1S concentrations during applications of Bio-E-Gel alone and in combination with sunscreen. The serum hormone levels and PK parameters were determined following application

of only Bio-E-Gel and also when sunscreen was applied either 10 min before or 25 min after the application of Bio-E-Gel.

Safety Evaluation:
Hematology, chemistry, urinalysis, and a brief physical examination (including vital signs) were performed during screening, and on Day 45. Adverse events were assessed prior to each drug application, and for 6 days (Day 50) following the last application.

Results:

Bio-E-Gel administered alone (A) and sunscreen application 10 min before Bio-E-Gel (B):

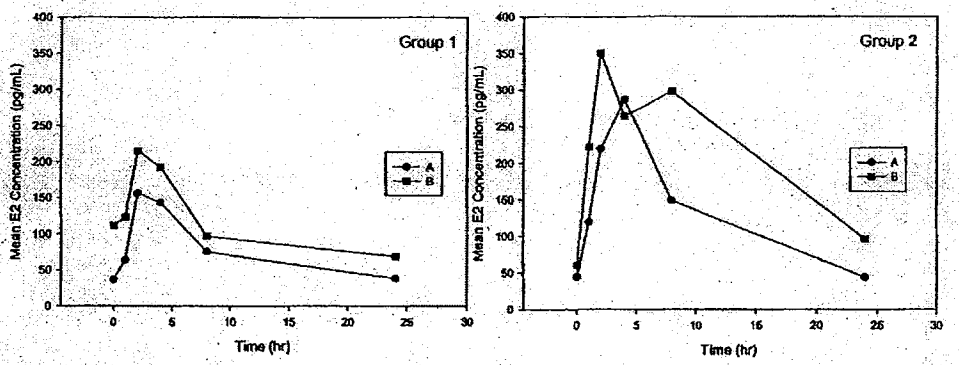
The concomitant application of sunscreen (10 min before Bio-E-Gel) with Bio-E-Gel increased the time averaged steady-state serum E2 concentrations (C_{ave}) from a mean of 100.8 pg/mL to 159.8 pg/mL. The C_{ave} mean ratio (B/A) was approximately 155%. The E2 exposure (AUC_{0-24}) increased from a steady-state mean of 2420 pg*h/mL (A) to a steady-state mean of 3835 pg*h/mL after 7 days of dual Bio-E-Gel and sunscreen (10 min before) application (B). As seen with C_{ave}, the AUC_{0-24} mean ratio (B/A) was approximately 155%. The mean maximum E2 serum concentrations (C_{max}) achieved following 15 days of Bio-E-Gel administration alone was 225 pg/mL versus a steady-state mean of 333 pg/mL after dual application of Bio-E-Gel and sunscreen, with a C_{max} mean ratio (B/A) of approximately 157%. The ratios mentioned above and listed in Table 34 are mean of individual ratios and were very similar to the ratios calculated by dividing group means.

Table 34: Estradiol PK Parameters and Mean Ratios (%) for All Subjects After Bio-E-Gel Alone (A) was Administered Daily for 15 Days, Followed by Sunscreen Administered 10 Min Prior to Bio-E-Gel (B) Daily for 7 Days

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

Period A: Steady-state period when Bio-E-Gel was administered alone
Period B: Steady-state period when sunscreen was applied 10 min prior to Bio-E-Gel
* Ratio (B/A) was calculated for each subject as the parameter value under B divided by the parameter value under A. The descriptive statistics summarize these ratios across all subjects.

Figure 33: Mean serum E2 concentrations by treatment and group



Group 1 (n = 6): Day 15 (A: Bio-E-Gel Administered Alone) and Day 22 (B: Bio-E-Gel and sunscreen application 10 min before)

Group 2 (n = 5): Day 37 (A: Bio-E-Gel Administered Alone) and Day 44 (B: Bio-E-Gel and sunscreen application 10 min before)

The serum E2 level in the crossover period is much higher than that in the initial period as can be readily seen in the plot above. The AUC_{0-24} for treatment A (Bio-E-Gel alone, group 2) on day 37 was 3194 ± 1935 pg*h/mL, a 1.8-fold increase compared to same treatment on day 15 (group 1) (AUC_{0-24} of 1775 ± 503 pg*h/mL). Note that this is a cross-group comparison. However, the alternate cross-group comparison and combined cross-over comparison yielded similar results (see below).

Bio-E-Gel administered alone (C) and sunscreen application 25 min after Bio-E-Gel (D):

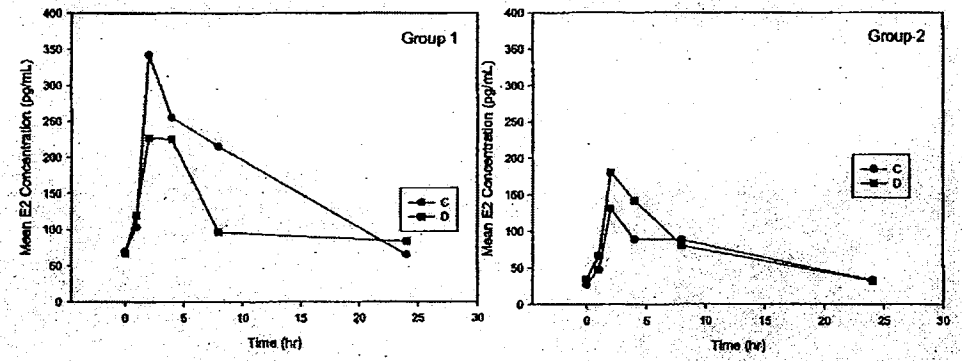
E2 exposures when sunscreen was applied 25 min after the application of Bio-E-Gel were apparently not significantly different from Bio-E-Gel applied alone. The concomitant application of sunscreen with Bio-E-Gel did not appear to increase C_{ave}. The mean AUC_{0-24} appeared to be decreased slightly from a mean steady-state of 3043 pg*h/mL to a steady-state mean of 2361 pg*h/mL after 7 days of daily dual Bio-E-Gel and sunscreen (+25 min) application, or a reduction of about 22% with sunscreen. The ratio of the geometric means was 0.93. However, the mean of individual AUC (D)/(C) ratios was 1.03 (Table 35). The mean C_{max} achieved following 15 days of Bio-E-Gel administration alone was approximately 326 pg/mL versus a steady-state mean of 228 pg/mL after dual application of Bio-E-Gel and sunscreen, resulting in a C_{max} mean ratio (D/C) of approximately 0.70, or a 30% reduction with sunscreen. The ratio of the geometric means was 0.88. The mean of individual ratios was 1.08 (Table 35).

Table 35: Estradiol PK Parameters and Mean Ratios (%) for All Subjects After Bio-E-Gel Alone (C) was Administered Daily for 15 days Followed by Sunscreen Administered 25 Min After Bio-E-Gel (D) Daily for 7 Days

PK parameter	Descriptive Statistics	C	D	Individual Subject D/C Ratios[a] (%)
C_{ave} (pg/mL)	N	11	11	11
	Mean	126.8	98.4	102.9
	SD	110.3	37.8	43.5
	CV%	87.0	38.4	42.3
	GeoMean	95.5	88.8	93.0
AUC_{0-24} (pg·h/mL)	N	11	11	11
	Mean	3043.1	2361.2	102.9
	SD	2646.5	906.3	43.5
	CV%	87.0	38.4	42.3
	GeoMean	2291.1	2130.5	93.0
C_{max} (pg/mL)	N	11	11	11
	Mean	325.9	228.1	108.3
	SD	323.8	111.5	61.0
	CV%	99.4	48.9	56.4
	GeoMean	212.1	187.6	88.4

Period C: Bio-E-Gel steady-state period when Bio-E-Gel was administered alone
Period D: Steady-state period when sunscreen was applied 25 min after Bio-E-Gel
[a] Ratio (D/C) was calculated for each subject as the parameter value under D divided by the parameter value under C. The descriptive statistics summarize these ratios across all subjects.

Figure 34: Mean serum E2 Concentration by treatment and group



Group 1 (n = 6): Day 37 (C: Bio-E-Gel Administered Alone) and Day 44 (D: Bio-E-Gel and sunscreen application 25 min after)

Group 2 (n = 5): Day 15 (C: Bio-E-Gel Administered Alone) and Day 22 (D: Bio-E-Gel and sunscreen application 25 min after)

The serum E2 level in the crossover period (left panel) again is much higher than that in the initial period. The AUC_{0-24} for treatment C (Bio-E-Gel alone, group 1) on day 37 was 4084 ± 3104 pg*h/mL, a 2.3-fold increase compared to same treatment on day 15 (group 2) (AUC_{0-24} of 1795 ± 1375 pg*h/mL). Note that this is also a cross-group comparison.

When all data for Bio-E-Gel alone (i.e., both treatment Groups A and C) were used, the AUC_{0-24} on day 15 was 1784 ± 940 pg*h/mL and on day 37 was 3679 ± 2556 pg*h/mL, a 2.1-fold increase in the crossover period versus the initial period ($p<0.05$ Student's t test). The Day 37:Day 15 ratio for geometric means is 3114 pg*h/mL:1572 pg*h/mL, or about 2-fold increase on Day 37. Other PK studies (EST007 and EST003) have suggested that steady state should be reached well prior to 15 days of dosing.

It is not clear why there is this significant increase on Day 37 relative to Day 15. Estradiol has been shown to cause an increase in level of sex hormone binding glubulins (SHBG) that may lead to increased total serum estradiol due to its binding to SHBG. However, transdermal application of estradiol has been generally reported to not significantly affect SHBG level. Additionally, in this NDA, study EST003 showed that SHBG level only increased slightly from a baseline level of 72.5 nM to 77.83 and 88.83 nM after 7 and 14 daily applications of 1.25 g/day Bio-E-Gel. Similarly, study EST005 showed that circulating SHBG serum concentration were increased slightly compared to placebo in the Bio-E-Gel 0.87 g/day and 1.7 g/day dose groups and were statistically significantly increased in the Bio-E-Gel 2.6 g/day dose group beginning at week 4. However, all mean trough levels of SHBG (85 – 111 nM) were similar to placebo group (~80 nM) and fell within the normal range (40 – 120 nM).

Could the prolonged application of Bio-E-Gel and/or sunscreen affect the absorption of Bio-E-Gel? It is probably not due to Bio-E-Gel alone as in the clinical phase 3 study, trough levels were measured at 4, 8, and 12 weeks and steady state appeared to be reached by week 4. By deduction, it appears that the 7 days of sunscreen application on days 16 – 22 may have led to increase Bio-E-Gel bioavailability on day 37. The mechanism of this proposed phenomenon is not known.

Estrone and Estrone sulfate PK:
Similar patterns were observed with E1 and E1S as was seen with E2 (i.e., 1.) sunscreen application 10 min prior caused increased drug exposure, 2.) sunscreen application 25 min after did not have significant effect with group 1 having slight decrease and was similar in group 2, and 3.) exposure in the crossover period (days 37 and 44) were higher than the initial period (days 15 and 22).

Estradiol/Estrone ratio in subjects given Bio-E-Gel only on Day 15:
As indicated above the estradiol AUC_{0-24} on Day 15 for all 11 subjects given Bio-E-Gel alone was 1784 ± 940 pg*h/mL. The estrone AUC_{0-24} in these same subjects was 1370 ± 529. The ratio of mean estradiol AUC/mean estrone AUC is 1.3. Mean of individual AUC ratios is 1.33 ± 0.56. The treatment target estradiol/estrone ratio is about one.

Conclusions:
When the sunscreen was applied 10 min before the application of Bio-E-Gel, there were increases in E2, E1, and E1S average serum concentrations of approximately 55%, 34% and 36%, respectively. Statistical analyses showed that treatment and period of sunscreen application were confounded. When sunscreen was applied 25 min after Bio-E-Gel application, there were no clinically significant changes in the E2, El, and E1S average serum concentrations. Exposure (AUC) to E2 in Bio-E-Gel only subjects was[ ]-fold higher on Day 37 compared to Day 15, where sunscreen was also applied on days 16 – 22. Based on these results, it is recommended that occasional sunscreen users should not apply sunscreen to the same site prior to Bio-E-Gel application and only apply sunscreen at least 25 minutes after Bio-E-Gel application. Clinical judgement should be applied if daily application of sunscreen to site of Bio-E-Gel application is needed due to potential increase in serum concentration. No medically important AEs, including skin irritation, were observed during this study.

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/s/

Doanh Tran
10/5/2006 01:11:17 PM
PHARMACOLOGIST

Ameeta Parekh
10/16/2006 04:11:54 PM
BIOPHARMACEUTICS

OFFICE OF CLINICAL PHARMACOLOGY - REVIEW ADDENDUM

NDA: 21-813	Submission Date(s): 02/16/2006
Brand Name	Elestrin®
Generic Name	Estradiol gel 0.06%
Reviewer	Doanh Tran, Ph.D.
Acting Team Leader	Myong-Jin Kim, PharmD
OCP Division	Division of Clinical Pharmacology 3
OND division	Division of Reproductive and Urologic Products
Sponsor	BioSante Pharmaceuticals, Inc.
Relevant IND(s)	IND 51,229
Submission Type; Code	Original NDA (3S)
Formulation; Strength(s)	Topical gel, 0.06% w/w
Indication	Treatment of moderate-to-severe VMS

NDA 21-813 was submitted on 02/16/2006 with the proposed brand name Bio-E-Gel®. There were / doses of 0.87, 1.7 [ ] g/day for / proposed indications:

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

[ ]

The Office of Clinical Pharmacology Review was filed in DFS on 10/5/2006 with final signatory sign off on 10/16/2006.

The following changes have been made since the original review:

1. The brand name has been changed to Elestrin® and was accepted by the review team.
2. The NDA has been [ ] as follow:
   a. NDA 21-813 contains doses of 0.87 and 1.7 g/day and a single indication of treatment of moderate-to-severe vasomotor symptoms associated with menopause.

[ ]

The Office of Clinical Pharmacology has no pending issues. This reviewer finds NDA 21-813 acceptable from a clinical pharmacology perspective.

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/s/

Doanh Tran
12/14/2006 03:11:43 PM
PHARMACOLOGIST

Myong-Jin Kim
12/14/2006 03:29:50 PM
PHARMACOLOGIST

Office of Clinical Pharmacology
New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-813	Brand Name	Bio-E-Gel
OCP Division (I, II, III, etc.)	DCP3	Generic Name	Estradiol topical gel 0.06%
Medical Division	DRUP	Drug Class	Hormone
OCP Reviewer	Doanh Tran, Ph.D.	Indication(s)	Hormone replacement therapy
OCP Team Leader	Ameeta Parekh, Ph.D.	Dosage Form	Topical gel
		Dosing Regimen	0.87 –[ ]g once daily
Date of Submission	02/16/2006	Route of Administration	Topical
Estimated Due Date of OCPB Review	10/16/2006	Sponsor	BioSante Pharmaceuticals, Inc.
PDUFA Due Date	12/15/2006	Priority Classification	Standard
Division Due Date	10/16/2006		

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			RIA assay in appendix 16.1.10 or 16.1.13
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	1		Study EST006, PK in the male partner only to assess transfer by direct contact
multiple dose:	x	5		Studies EST003, EST007, and EST008 used the TBM formulation. The other [ ] studies [ ] used a different formulation that was not bridged.
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:	x			Information from PK studies above
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:	x	1		Study EST004
Phase 3:	x	1		Study EST005
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				A BE study is not needed since the phase 2, 3, and most phase one studies used the to-be-marketed formulation.
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:	x	1		Release rate criteria were proposed. See 6.9.1 and 6.9.2
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		9		

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)	**Sunscreen and topical cream effect** **Dose response** **Minimal effective dose**	

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

Filing Memo

Clinical Pharmacology Review

NDA: 21-813
Compound: Bio-E-Gel™ (Estradiol Topical Gel 0.06%)
Sponsor: BioSante Pharmaceuticals, Inc.

Date: 2/27/2006
Reviewer: Doanh Tran

Background: Estradiol is available for estrogen replacement therapy in the United States in oral formulations, vaginal ring or vaginal preparations, and preparations for percutaneous absorption in the form of a transdermal patch, topical emulsion, or hydroalcoholic gel. The goal of estrogen therapy is to provide relief of vasomotor and vulvovaginal symptoms in postmenopausal women. Theoretically, symptom relief would occur when estradiol concentrations are greater than those of postmenopausal women with symptoms and similar to the premenopausal hormonal milieu.

The sponsor has submitted an NDA for estradiol topical gel 0.06% (Bio-E-Gel™) seeking indications of treatment of moderate-to-severe vasomotor symptoms associated with menopause (VMS) [ ] [ ] The proposed dosage regimen is to start with 0.87 g of gel, which contains 0.52 mg of estradiol, daily (one pump) applied to the upper arm. Other doses include 1.7 g (2 pumps) [ ] daily.

The sponsor conducted / PK studies. However, only 4 studies (EST003, EST006, EST007, and EST008) used the same formulation in the 2 clinical trials supportive of the proposed indication (EST004 and EST005) and that is the formulation planned for commercialization. The other / PK studies [ ] used a Bio-E-Gel [ ] formulation that is different than the clinical trial formulation and are included for completeness (only a summary was provided for [ ] /) but not for support of the NDA.

Two studies (EST003 and EST007) measured serum concentrations of estradiol and its metabolites following topical application of Bio-E-Gel for 14 days in postmenopausal women. Pharmacokinetic parameters for estradiol and its metabolites were derived from the measured values as well as values that were adjusted for baseline concentrations of endogenous hormones. Adjusted PK parameters provide an estimate of the absorbed drug. Study EST006 evaluated the removal of Bio-E-Gel by washing and the potential transfer of Bio-E-Gel by skin-to-skin contact and study EST008 evaluated the effect of sunscreen on the absorption of Bio-E-Gel.

Absorption: According to the sponsor, steady state concentrations of estradiol are achieved in approximately 3 days. Following 14 daily doses of 0.87 g Bio-E-Gel the mean unadjusted estradiol AUC0-24 was 335.2 pg*hr/mL, mean Cmax of 21.6 pg/mL, and median Tmax of 18 hours (range 1-20 hours). Application of sunscreen 10 minutes before application of Bio-E-Gel increased the mean exposure by approximately 55% with individual increases as high as / %. No significant change in mean estradiol exposure was observed when sunscreen was applied 25 minutes after application of Bio-E-Gel. There appear to be a period effect in the sunscreen study, where subjects in the Bio-E-Gel alone group had mean AUC on day 37 that was [ ] fold higher than on day 15. Generally, exposure in the second crossover period (i.e., days 37 and 44) was higher than the first period regardless of treatment group. It is not clear what was the cause of this significant increase in exposure.

Distribution, metabolism, excretion, and drug-drug interactions: The sponsor is relying on current knowledge base to address these areas for estradiol. This reviewer concurs that there are sufficient information in other estradiol product labels to enable labeling of Bio-E-Gel.

Special population: Bio-E-Gel pharmacokinetics was studied in postmenopausal women, which is the target population. No study was conducted in patients with renal or hepatic impairment or any other special population.

Estradiol transfer: Less than 10% of the estradiol dose was measured on the skin via cotton swabs at 2 and 8 hours after application. Direct arm to arm contact with male partner for 5 minutes at 2 and 8 hours after application did not result in significant changes in estradiol level in the male partner. Washing of the application site at 8 hours after application reduced the residual estradiol to 1% of applied dose.

Clinical vs. to-be-marketed formulation: The sponsor stated that the clinical and the to-be-marketed formulation are the same.

Recommendation:

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 3 finds that the Human Pharmacokinetics and Bioavailability section for NDA 21-813 is fileable.

Comments for sponsor:

1. Regarding study EST008, we are concerned with the increased estradiol exposure in the group where Bio-E-Gel was applied after sunscreen (mean increase of 55% with individual increase as high as /% relative to Bio-E-Gel alone) and the increased estradiol exposure in all groups in the second crossover period (mean increase of [ ] fold relative to the first period).
    - [redacted]
    - Please provide rationale for the higher exposure to estradiol in the second crossover period (i.e., days 37 and 44) as compared to the first period (i.e., days 15 and 22) in study EST 008. Specifically, please address whether this was related to the application of sunscreen on days 16 – 22 or other factors that may be responsible for this observation (e.g., change in SHBG and estradiol binding).
2. The nominal delivery rate estimate for the 2.6 g dose appears to be low. For calculation of the nominal delivery rate for the 2.6 g dose, you used data from study EST003, where 2.5 g of gel was applied to the front and inner thigh area, to estimate a nominal delivery rate of 0.064 mg/day. The mean unadjusted average estradiol concentration (C_{ave}) in this study was 53.4 pg/ml. We noted that in study EST008, where 2.6 g was applied to the upper arm (i.e., same dose and application site as

in the proposed label), the mean unadjusted C_{ave} for estradiol on day 15 were 74 and 75 pg/ml for group 1 and 2, respectively. Considering baseline mean estradiol levels of 4 – 8.1 pg/mL in your studies EST007 and EST003 and applying the same equation that you used, the estimate nominal delivery rate would be approximately in the range of 0.084 to 0.091 mg/day. [ ]

[ ]

Doanh Tran, Ph.D., Primary Reviewer — Date

Ameeta Parekh, Ph.D., Team Leader — Date

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/s/

Doanh Tran
4/10/2006 03:57:57 PM
PHARMACOLOGIST

Ameeta Parekh
5/3/2006 04:34:38 PM
BIOPHARMACEUTICS