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APPLICATION NUMBER:

208470Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 208470
Supporting document/s:	IND 78027
Sponsor's letter date:	October 16, 2015
CDER stamp date:	October 16, 2015
Product:	Intrarosa [prasterone; dehydroepiandrosterone (DHEA)]
Indication:	Indicated for the treatment of moderate to severe dyspareunia, a symptom of vulvovaginal atrophy due to menopause.
Sponsor:	Endoceutics, Inc.
Review Division:	DBRUP
Reviewer:	Alex Jordan, PhD
Supervisor/Team Leader:	Summan Mukesh, PhD, DABT
Division Director:	Hylton Joffe, MD, MMSc
Project Manager:	Kimberly Shiley, RN

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1 Executive Summary

1.1 Introduction

NDA 208470 was submitted by Endoceutics Inc. for Intrarosa [prasterone; dehydroepiandrosterone (DHEA)] in October 2016. Prasterone is a steroid synthesized in the adrenals which acts primarily as a precursor to androgens and estrogens. It is indicated for the treatment of moderate to severe dyspareunia, a symptom of vulvovaginal atrophy due to menopause.

1.2 Brief Discussion of Nonclinical Findings

All pivitol nonclinical studies were conducted using oral administration of the drug, which differs from the clinical (intravaginal) exposure route, and in accordance with US FDA GLP (21CFR58), as stated by the sponsor. Safety margins to the expected human exposure were estimated in postmenopausal women using intravaginal DHEA $AUC_{0\text{-}24h}$ value of 56 ng.h/ml.

Prasterone [dehydroepiandrosterone (DHEA)] and its sulfated metabolite (DHEA-S) are the most abundant steroids in the body. DHEA is produced in the adrenals and is the biosynthetic precursor to the sex hormones testosterone and estradiol.

Vaginal and percutaneous administration of DHEA prevents the decreased weight and histological signs of vaginal atrophy induced by ovariectomy in rats.

In 6 month rat and 12 month monkey toxicology studies with oral administration, DHEA was essentially non-toxic and produced no adverse effects in monkeys at doses up to 10 mg/kg (7-12 times human exposure based on AUC). In rats there were some estrogen/androgen related effects including minimal to slight squamous metaplasia of the glandular epithelium of the uterus at doses of 10 and 100 mg/kg (0.2-16 times human exposure).

DHEA was negative in three genotoxicity studies; bacterial mutagenesis assay (Ames test), in vitro chromosomal aberrations assay with human peripheral blood lymphocytes, and in vivo mouse bone marrow micronucleus assay.

No reproductive studies were performed with DHEA since it is indicated solely for postmenopausal women. It is contraindicated in pregnancy.

No carcinogenicity studies were performed with prasterone. This is an endogenous non-genotoxic steroid and the systemic concentrations achieved in post-menopausal women taking the drug are equal or less than the endogenous concentrations seen in younger women. Vaginal concentrations of DHEA will be increased but the concentration of the active hormone, estrogen, will be no higher than the estrogen concentration achieved in women taking approved vaginal estrogens. According to the International Agency for Research on Cancer (IARC, member of the World Health Organization), “post-menopausal estrogen therapy is carcinogenic to humans”. Furthermore, IARC states: “there is sufficient evidence for the carcinogenicity of testosterone in experimental animals and in the absence of adequate data in humans, it is reasonable, for practical purposes, to regard testosterone as if it presented a carcinogenic risk to humans”.

1.3 Recommendations

1.3.1 Approvability

Intrarosa is approvable.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

INDICATIONS AND USAGE
Intrarosa is a steroid indicated for the treatment of moderate to severe dyspareunia, a symptom of vulvar and vaginal atrophy due to menopause.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Risk Summary
Animal data on pregnancy risk are not available

8.2 Lactation –
Risk Summary
Animal data on lactation are not available

8.4 Pediatric Use – Animal data on pediatric use are not available. .

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis
Long-term studies in animals to evaluate carcinogenic potential have not been conducted with prasterone. Two metabolites of prasterone, testosterone and estradiol, are carcinogenic in animals.

Mutagenesis
Prasterone was not genotoxic in the in vitro bacterial mutagenesis assay (Ames test), the in vitro chromosomal aberrations assay with human peripheral blood lymphocytes and in vivo in the mouse bone marrow micronucleus assay.

Fertility
Fertility studies were not conducted with prasterone.

2 Drug Information

2.1 Drug

CAS Registry Number

53-43-0

Generic Name

Prasterone

Code Name

EM-760

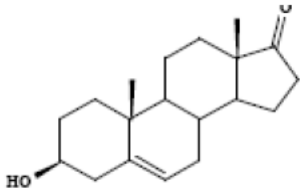
Chemical Name

3β-hydroxy-5-androsten-17-one or 3β-hydroxyandrost-5-en-17-one

Molecular Weight

288.4

Structure or Biochemical Description



Established Pharmaceutical Classes (EPC)
Steroid

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 78,027; [redacted]

2.3 Drug Formulation

Table 1: Composition of the Drug Product

Component and Quality Standard (and Grade, if applicable)	**Function**	**Strength (label claim)**
		6.5 mg DHEA/ [redacted]
		Quantity per unit (mg)
Prasterone, in house	Drug Substance	6.5
Hard Fat, NF (Witepsol [redacted])	[redacted]	[redacted]
Total (mg):		

2.4 Comments on Novel Excipients

Witepsol is a blend of synthetic triglycerides. It is commonly used in compounding and has been used as a [redacted] in several FDA approved products including miconazole nitrate (vaginal), canesa (rectal) and prostin E2 (vaginal).

2.6 Proposed Clinical Population and Dosing Regimen

Intrarosa in indicated for use in postmenopausal women with vulvovaginal atrophy. Intrarosa is a [redacted] containing 6.5 mg of DHEA to be administered intravaginally once daily. The drug is not intended to be used during pregnancy and no reproductive toxicology studies were conducted.

2.7 Regulatory Background

The filing review by Kim Hatfield, PhD, summarized the regulatory background of Intrarosa as follows:

The IND for the product and indication (IND 78027) was submitted in 2007. The initial IND contained a 1 year monkey toxicology study, a 6 month rat toxicology study, pharmacology, pharmacokinetics and 3 genotoxicity studies. No reproductive toxicity studies were conducted and none were needed due to the indicated population of postmenopausal females. In addition, the requirement for carcinogenicity studies was waived, as long as the sponsor agreed to include language for carcinogenicity that exists in estrogen and androgen product labeling (e.g. Premarin vaginal cream, or topical androgens such as Androgel and Fortesta). Refer to a review and waiver of in vivo carcinogenicity studies (Agency letter dated 1/24/2014 for IND (b) (4), an IND for (b) (4)

In a meeting on April 30, 2009, it was agreed that the findings reported in the completed repeated-dose one year oral toxicity study with DHEA in monkeys, the 26 week oral toxicity study in rats, and the three standard genotoxicity assays are adequate to support the submission of a NDA for 0.5% (6.5 mg) DHEA for a vaginal atrophy indication.

In February 2013, the Sponsor submitted a request for waiver of carcinogenicity studies to IND (b) (4), a second IND for DHEA submitted by Endoceutics for the (b) (4) In a letter sent to the sponsor on January 24, 2014, the Division concluded:

> "We agree that no carcinogenicity studies are needed for DHEA. In vivo carcinogenicity studies can be waived for DHEA vaginal (b) (4) provided the Sponsor agrees to add wording regarding the carcinogenicity of estrogens and androgens in the product labeling. This wording would be similar to product labeling for Premarin vaginal cream and topical androgens such as Androgen and Fortesta.

At the pre-NDA meeting on April 27, 2015, the following were communicated:

All nonclinical requirements have been met to support the NDA.

All nonclinical study reports that will be used to support the nonclinical portion of your current NDA submission should be resubmitted in Module 4 of the NDA.

A tabular listing should be provided of titles of studies from all IND's or NDA's that you intend to use to support the nonclinical portion of your current NDA submission.

The proposed prescribing information for your product, specifically under Section 8, will need to conform to the Pregnancy and Lactation Labeling Rule (PPLR).

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology, pharmacokinetics, 1 year oral monkey toxicology study, 6 month oral rat toxicology study and three genotoxicity studies were reviewed.

3.2 Studies Not Reviewed

None.

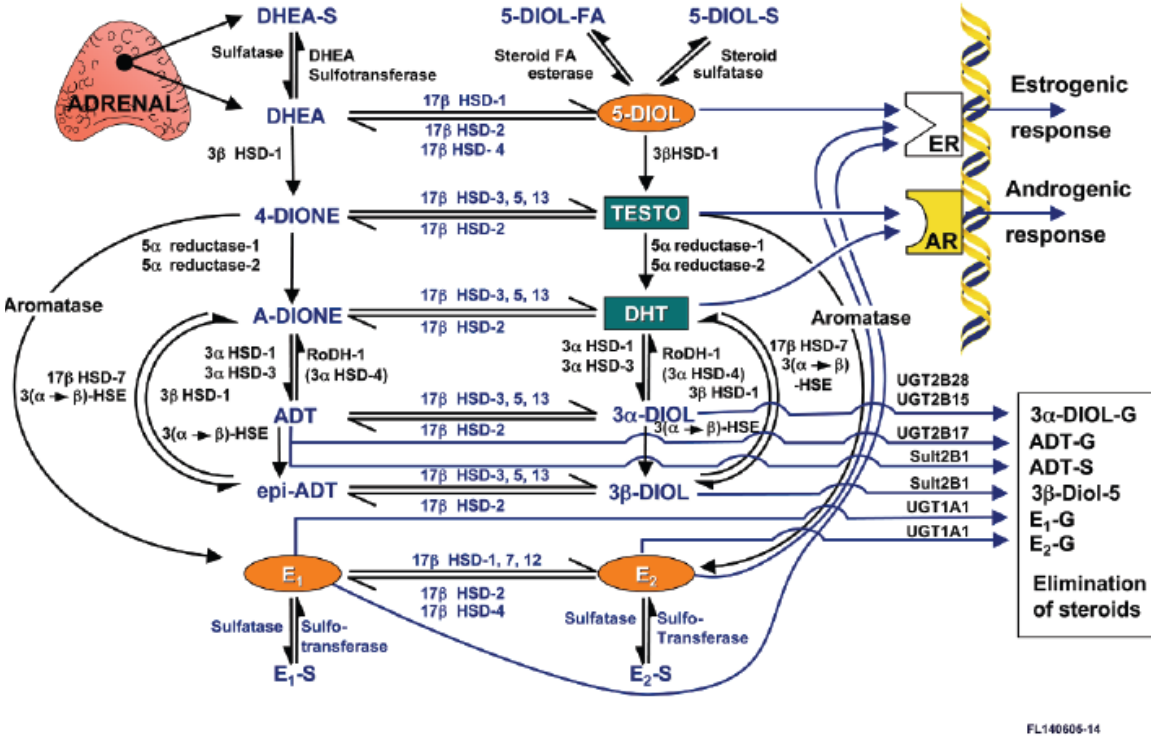
3.3 Previous Reviews Referenced

IND 78, 027; IND (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

Dehydroepiandrosterone (DHEA) and DHEA-S (sulfated metabolite of DHEA) are endogenous hormones secreted mainly by the zona reticularis of the adrenal cortex of primates. DHEA is formed through metabolism of pregnenolone by CYP17 and further metabolised to androstenedione and androsterone, or testosterone and estradiol in the peripheral tissues that contain the enzymes 3-beta hydroxysteroid dehydrogenase/delta 5-4 isomerase, 17-beta hydroxysteroid dehydrogenase and aromatase. DHEA itself has weak androgenic effects via the androgen receptor. DHEA-S is the more abundant form in circulation with ~300X the level of DHEA. In humans, circulating levels of DHEA/DHEA-S peak at about age 20-30 and then decline in both genders to ~30% of peak levels at age 60-80.



2 Human Steroidogenic and Steroid-Inactivating Enzymes in Peripheral Intracrine Tissues.

DHEA/DHEA-S is the most abundant steroid in humans, and is synthesized in primates in the adrenals as opposed to other species in which circulating DHEA is not present. As such, data from studies conducted in non-primates may have limited relevance for humans. `

4.2 Secondary Pharmacology

Literature summary from Kim Hatfield, PhD

> DHEA acts mainly through the androgen receptor (AR) and estrogen receptor (ER), either directly or after being converted to androgen or estrogen. However, it can also act

via non-steroid receptor-associated non-genomic pathways and via other mechanisms (reviewed in Arnold and Blackman, 2005).

DHEA has both agonistic and antagonistic effects on the AR, but only at supra-physiological concentrations (Chen et al., 2005). By contrast, DHEA has agonistic effects on the ER, has a higher affinity for ERβ than ERα, and activates ERβ-mediated transcription at physiological nanomolar concentrations. Its androgenic or estrogenic effects (e.g. cardiovascular system, breast cancer cells in vitro) primarily depend on pre/postmenopausal status, gender, and tissue, among others (Ebeling and Koivisto, 1994).

DHEA has a number of different pharmacologic actions. It inhibits glucose-6-phosphate dehydrogenase (G6PDH) in vivo and in vitro, thereby causing oxidative stress and acting as a pro-oxidant (Leopold and Loscalzo, 2000). However, it can also act as an antioxidant as a metal chelator and inducer of hydroxyl radical scavenging (Tamagno et al., 1998). DHEA synthesized in the brain can act as a GABA receptor antagonist (Demirgoren et al., 1991) and can enhance the neuronal NMDA receptor response (Debonnel et al., 1996). In rabbits with experimental atherosclerosis, DHEA decreased plaque size by ~50%, but had a minimal effect on serum lipid levels (Gordon et al., 1983).

DHEA also has anti-obesity activity in mice. Long term treatment of genetically obese mice led to a significant reduction in growth without affecting food consumption, possibly due to reduced fat synthesis (Yen et al., 1977; Schwartz et al., 1981). Many studies have also shown that DHEA impairs mitochondrial energy production by inhibiting mitochondrial respiration and shifting energy metabolism from oxidative phosphorylation to glycolysis, resulting in less efficient ATP production (reviewed in Safiulina et al., 2006).

In ovariectomized (OVX) female rats treated intravaginally with DHEA suppositories (0.33 mg, 0.66 mg or 1 mg; 14 days), treatment-related pharmacological effects included 1) decreased body weight (1 mg; estrogenic effect), 2) mucification of the vaginal epithelium (all doses; androgenic effect), and 3) enlargement of the dorsal skin sebaceous glands (1 mg; androgenic effect) (Berger et al., 2008). There was no effect of intravaginal DHEA on the uterus or mammary gland.

DHEA had a beneficial effect on bone in estrogen-deficient female rats (30 mg dermal twice daily; 12 months) (Martel et al., 1998). DHEA reversed the OVX-induced decreases in femoral, spine and total body bone mineral content (BMC) and bone mineral density (BMD) and increased BMC and BMD values above those of intact animals. The increases were correlated with increased bone formation and decreased bone resorption.

Arnold and Blackman 2005 Endoc 146:4565-67.
Berger etal, 2008 Steroid Biochem Mole Biol 109:67-80.
Chen etal, Endoc 146:4568-76 (from Arnold and blackman 2005).
Debonnel etal, 1996 J Endoc 150:S33-42.
Demirgoren etal, 1991 Neurosci 45:127-35.
Ebeling and Koivisto 1994 Lancet 343:1479-81.

Leopold and Loscalzo 2000 Am J Physiol Heart Circ Physiol 279:H2477-85.
Martel etal, 1998 J Endoc 157:433-42.
Safiulina etal, 2006 Toxicol Sci 93:348-56.
Schwartz etal, 1981 Nutrition and Cancer 3:46-53.
Tamagno etal, 1998 Cell Biochem Funct 16:57-63.
Yen etal, 1977 Lipids 12:409-13.

4.3 Safety Pharmacology

No studies submitted by Sponsor. There was some information in the literature summarized by Kim Hatfield, PhD.

Effects on the cardiovascular system
In Wistar rats, 30 mg/kg DHEA-S inhibited the increase in blood pressure resulting from repeated but not single stress exposures (Obut, et al., 2013). In an infant rodent model of pulmonary hypertension, DHEA reduced pulmonary artery pressure, pulmonary artery remodeling and right-ventricular hypertrophy (Dumas de la Roche et al., 2013).

Effects on the central nervous system
In male mice, DHEA (0.6% in diet; 10 weeks) lowered neuronal density in primary motor cortex and hippocampus. Motor function of rats was impaired 4 weeks after discontinuing DHEA administration (Safiulina et al., 2006). In the rat, a single dose of 20 mg/kg DHEA given more than 3 hrs after transient cerebral ischemia was neuroprotective, but when given 1 hr before or after ischemia, it exacerbated ischemia-induced neuronal death and learning impairment (Li et al., 2009).

Dumas de la Roche etal, 2013 Pediatr Res 74:163-9.
Li etal, J Cerebral Blood Flow and Metab 2009 29:287-96.
Obut etal, 2013 Bull Exp Biol Med 156:35-7.
Safiulina etal, 2006 Toxicol Sci 93:348-56.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Plasma levels of DHEA, 5-DIOL and DHEA-S were measured 0.5, 1, 2 and 4 h after intravaginal administration of 0.33, 0.66 and 1 mg DHEA suppositories in ovariectomized rats.

As shown in the graphs below, intravaginal administration of DHEA resulted in measurable DHEA, 5-DIOL and DHEA-S in the rat plasma.

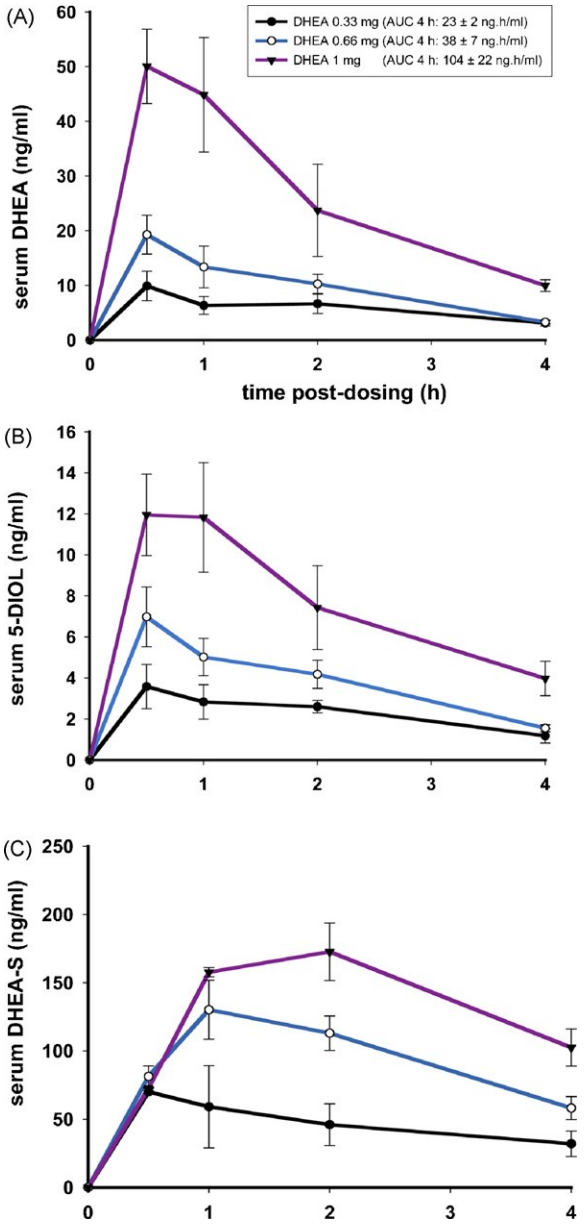


Fig. 4. Serum concentration of (A) DHEA, (B) 5-DIOL and (C) DHEA-S, 0.5, 1, 2 and 4 h following intravaginal application of a suppository containing 0.33, 0.66 or 1 mg DHEA in OVX rats. In (A), $p < 0.01$ between 1 mg and both 0.33 and 0.66 mg doses (except at 2 h post-dosing). In (B), $p < 0.05$ between 1 mg and 0.33 mg doses while $p < 0.05$ between 1 mg and 0.66 mg doses, at 1 and 4 h post-dosing. Finally, in (C) $p < 0.01$ and $p < 0.05$ between 1 mg and 0.33 or 0.66 mg doses, respectively (at 1, 2 and 3 h post-dosing), while $p < 0.05$ between 0.66 mg and 0.33 mg doses (at 2 h post-dosing).

Plasma levels of androstenedione, testosterone, dihydrotestosterone, estradiol and estrone were below detectable levels.

Human Toxicokinetics from study ERC-213

Area under the curve (ng.h/ml serum; 0-24h) on day 7 of daily administration of intravaginal DHEA (6.5 mg; 0.5%) suppositories in 40-75 year old postmenopausal women with vaginal atrophy

Group	DHEA	DHEA-S	Testo	DHT	E1	E2
Placebo	24.8	8350	2.6	0.6	0.30	0.07
DHEA (0.5%)	56.2	13290	2.8	0.9	0.37	0.10

Average serum steroid levels (ng/ml serum) of DHEA and metabolites on day 7 of daily administration of intravaginal DHEA (6.5 mg; 0.5%) suppositories to 40-75 year old postmenopausal women with vaginal atrophy

Group	DHEA	DHEA-S	Testo	DHT	E1	E2
Placebo	1.0	350	0.1	0.02	0.013	0.003
DHEA (0.5%)	2.3	550	0.1	0.04	0.015	0.004
Premenopausal women	4.5	1270	0.2	0.07	0.054	0.082

Postmenopausal women receiving DHEA (6.5 mg) had lower mean hormone levels than did premenopausal women.

6 General Toxicology

6.1 Single-Dose Toxicity

Not done.

6.2 Repeat-Dose Toxicity

Study title: 6-month study in male and female Sprague-Dawley rats

Key study findings: Little toxicity.

Study no.: 1376
Volume #, and page #: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: March, 2003
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: DHEA (EM-760), lot EM-760-13, 100% pure

Methods

Doses: 10 and 100 mg/kg
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 10/sex/gp

Route, formulation, volume, and infusion rate: oral gavage, suspension in 0.4% aqueous methocel A15 premium (methylcellulose)
Satellite groups used for toxicokinetics or recovery: 48/sex/gp for day 1; 18/sex/gp for day175.
Age: 7 wks
Weight: 149-288 g
Sampling times:
Unique study design or methodology (if any): In addition to the two gps receiving DHEA alone, there was another gp that received 10 mg/kg DHEA + 3 mg/kg estradiol or 10 mg/kg DHEA + a selective estrogen modulator (SERM) EM 652 HCl (SCH 57068 HCl; Acolbifene).

26-Week Oral Toxicity Study of Dehydroepiandrosterone, EM-652·HCl and Estradiol in Male and Female Sprague-Dawley Rats (b) (4) Study No. 1376): **Study Design**

Group	Dosing Suspension	DHEA		Estradiol		EM-652·HCl		Dose Volume (mL/kg)	Number of Rats					
									Males			Females		
		Total Daily Dose (mg/kg)	Dose Conc[a] (mg/mL)	Total Daily Dose (mg/kg)	Dose Conc[a] (mg/mL)	Total Daily Dose (mg/kg)	Dose Conc[a] (mg/mL)		Toxicity	Toxicokinetic Day 175	Toxicokinetic Day 1	Toxicity	Toxicokinetic Day 175	Toxicokinetic Day 1
1	Vehicle[b]	0	0	0	0	0	0	4	10	18	48	10	18	48
2	DHEA	10	2.5	0	0	0	0	4	10	18	48	10	18	48
3	DHEA	100	25	0	0	0	0	4	10	18	48	10	18	48
4	DHEA and E_2 Mixture	100	25	3	0.75	0	0	4	10	18	48	10	18	48
5	DHEA, E_2 and EM-652·HCl Mixture	100	25	3	0.75	120 or 600[c]	30 or 150[d]	4	10	18	48	10	18	48

a: Concentration.
b: 0.4% (w/v) aqueous methylcellulose.
c: Male rats received 120 mg EM-652·HCl/kg and female rats received 600 mg EM-652·HCl/kg except as described in Appendix 1.
d: Male rats received 30 mg EM-652·HCl/mL and female rats received 150 mg EM-652·HCl/mL except as described in Appendix 1.

Results:

Mortality: no test article related deaths

Clinical signs: no signs in animals receiving DHEA alone

Body weights: no change in females. There was an 8% reduction in BW and a

15% reduction in BW gain in HD males. No effect in LD males

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: not done

Hematology: no changes in animals receiving DHEA alone

Clinical chemistry: Decrease of 48% and 40% in total bilirubin values in HD females at study wks 14 and 27, respectively. Mild (less than 2X) increase in alk phos in HD males and females.

Urinalysis: No DHEA alone related effects

Gross pathology: 0/10, 1/10, 2/10 ovarian cysts in control, 10 mg/kg and 100 mg/kg, respectively.

Organ weights (specify organs weighed if not in histopath table):
Higher liver wts were observed in male and female rats dosed with 100 mg/kg but there were no histo changes (7.3, 8.1, 10.9 g for control, LD and HD females, respectively; 13.1, 13.6, 16.3 g for control, LD and HD males, respectively).

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (), no (x)

In DHEA treated rats, microscopic changes were observed in the adrenals of males, uterus and vagina of females. Minimal to slight diffuse vacuolation of adrenal cortical cells was seen in HD males. Minimal to slight squamous metaplasia of the glandular epithelium of the uterus was noted in 0/10, 4/10 and 3/10 female rats dosed with 0, 10 and 100 mg/kg. Minimal to moderate abnormal mucification of the vaginal epithelium was noted in 2/10 controls, 2/10 low dose and 5/10 HD females. The squamous metaplasia seen in the uterus was completely abolished with the addition of the SERM, EM-652, suggesting that the effect was most likely an estrogenic effect.

Table 42 Histopathologic Findings in Female Rats Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Organ	Finding	Control	DHEA (mg/kg/day)		DHEA (100 mg/kg/day) and Estradiol (3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			10	100	0	600
Estrous Cycle	Metestrus	1/10	0/10	0/10	0/11	0/10
	Diestrus: Persistent	0/10	0/10	0/10	0/11	10/10
	Estrus	5/10	6/10	5/10	4/11	0/10
	Estrus: Persistent	2/10	2/10	5/10	7/11	0/10
	Proestrus	2/10	2/10	0/10	0/11	0/10
Liver	Eosinophilic Cell Focus	0/10	0/10	0/10	7/11	0/10
Mammary Gland	Ectasia: Ducts and/or Alveoli	1/10	1/10	0/10	4/11	0/10
	Lobuloalveolar Development	0/10	0/10	0/10	0/11	9/10
	Proliferation: Tubular	0/10	3/10	1/10	11/11	0/10
Ovary	Cyst: Follicular	0/10	1/10	1/10	1/11	10/10
Pituitary	Vacuolation: Pars Distalis	0/10	0/10	0/10	0/11	10/10
Skin/Subcutis (Dorsal)	Hyper-keratosis	0/10	2/10	3/10	2/11	9/10
Uterus	Atrophy	0/10	0/10	0/10	0/11	10/10
	Metaplasia: Squamous	0/10	4/10	3/10	2/11	0/10
Vagina	Mucification: Epithelial, Abnormal	2/10	2/10	5/10	7/11	0/10

Table 43 Histopathologic Findings in Male Rats Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Organ	Finding	Control	DHEA (mg/kg/day)		DHEA (100 mg/kg/day) and Estradiol (3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			10	100	0	120
Adrenal	Vacuolation: Cortical	1/10	1/10	5/10	2/10	0/10
Epididymis	Oligo/ aspermia	0/10	0/10	0/10	8/10	0/10
Liver	Eosinophilic Cell Focus	2/10	2/10	0/10	9/10	0/10
Mammary Gland	Ectasia: Ducts and/or Alveoli	0/8	0/10	0/9	7/10	0/9
	Tubuloalveolar Development	0/8	0/10	2/9	10/10	3/9
Pituitary	Decreased Vacuolation: Pars Distalis	1/10	1/10	3/9	10/10	0/10
Prostate	Atrophy	0/10	0/10	0/10	9/10	0/10
Seminal Vesicle	Atrophy	0/10	0/10	0/10	10/10	0/10
Skin/Subcutis (Dorsal)	Atrophy (Epidermis)	0/10	1/10	0/10	8/10	0/10
Testis	Hypo/asper-matogenesis	0/10	0/10	0/10	8/10	0/10
Thymus	Atrophy: Lymphoid	0/10	1/10	0/10	3/10	0/10

Toxicokinetics:

Table 2 Serum Dehydroepiandrosterone Pharmacokinetic Parameter Values in Female Rats Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (100 mg/kg/day) and Estradiol (3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			10	100	0	600
AUC(0-24 hr) (ng·hr/mL)	Day 1	0	125	420	529	429
	Day 175	0	142	921	706	155
Cmax (ng/mL)	Day 1	0	31.9	68.6	61.4	69.0
	Day 175	0	29.1	247	385	45.1
Tmax (hr)	Day 1	NC	1.0	1.0	1.0	1.0
	Day 175	NC	1.0	1.0	1.0	1.0

NC = Not calculable

Table 4 Serum Dehydroepiandrosterone Sulfate Pharmacokinetic Parameter Values in Female Rats Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (100 mg/kg/day) and Estradiol (3 mg/kg/day)	
					EM-652·HCl (mg/kg/day)	
			10	100	0	600
AUC(0-24 hr) (ng·hr/mL)	Day 1	1	10377	187243	171269	213275
	Day 175	6	13670	166582	117156	9663
Cmax (ng/mL)	Day 1	0.94	4367	55457	40966	43196
	Day 175	0.72	5165	30521	32894	2522
Tmax (hr)	Day 1	1.0	1.0	1.0	1.0	2.0
	Day 175	12.0	1.0	1.0	1.0	1.0

Table 3 Serum Dehydroepiandrosterone Pharmacokinetic Parameter Values in Male Rats Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (100 mg/kg/day) and Estradiol (3 mg/kg/day)	
					EM-652·HCl (mg/kg/day)	
			10	100	0	120
AUC(0-24 hr) (ng·hr/mL)	Day 1	0	5	74	69	94
	Day 175	0	10	81	1161	52
Cmax (ng/mL)	Day 1	0	2.27	19.3	12.9	14.8
	Day 175	0.35	2.91	13.9	283	12.4
Tmax (hr)	Day 1	NC	1.0	1.0	1.0	2.0
	Day 175	3.0	1.0	2.0	1.0	2.0

NC = Not calculable

Table 5 Serum Dehydroepiandrosterone Sulfate Pharmacokinetic Parameter Values in Male Rats Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (100 mg/kg/day) and Estradiol (3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			10	100	0	120
AUC(0-24 hr) (ng·hr/mL)	Day 1	0	1168	11304	5154	7885
	Day 175	0	1327	3008	128766	2257
Cmax (ng/mL)	Day 1	0.71	895	1566	1080	1744
	Day 175	0	382	461	23678	516
Tmax (hr)	Day 1	0	1.0	4.0	1.0	2.0
	Day 175	NC	1.0	3.0	2.0	1.0

NC = Not calculable

Rats given 10 and 100 mg/kg DHEA orally for 175 days had DHEA $AUC_{0\text{-}24h}$ values of 142 and 921 ng.h/ml for females and 10 and 81 ng.h/ml for males. Humans given 0.5% (6.5 mg) DHEA in a vaginal suppository for 7 days had a mean serum DHEA $AUC_{0\text{-}24h}$ value of 56 ng.h/ml. Exposure margins are 3 and 16X for females and 0.2 and 1X for males.

The NOAEL was below 10 mg/kg unless one assumes the organ wt and histo effects were pharmacologic which they probably are (all the effects seen with DHEA have been seen in estrogen treated rats).

Study title: One-year oral toxicity study of DHEA, EM-652-HCl and estradiol in male and female cynomolgus monkeys

Key study findings: no toxicity seen
Study no.: 1375
Volume #, and page #: electronic
Conducting laboratory and location: (b) (4)

Date of study initiation: Feb. 2003
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: DHEA, lot EM-760-13, 99.7% pure

Methods

Doses: 2 and 10 mg/kg/day. Another two groups received 10 mg/kg DHEA with 0.3 mg/kg estradiol or 10 mg/kg DHEA with 0.3 mg/kg estradiol and 40 mg/kg EM-652 HCl (SERM).
Species/strain: cynomolgus monkeys
Number/sex/group or time point (main study): 4/sex/gp
Route, formulation, volume, and infusion rate: oral by nasogastric intubation, suspension in 0.4% aqueous methylcellulose at concentrations of 1, 5, and 150 mg/ml, vol of 2 ml/kg.
Satellite groups used for toxicokinetics or recovery: no
Age: young adult, males 4-6 yrs, females 6-18 yrs
Weight: 2.9 to 7.1 kg
Sampling times: blood was collected on days 1, 184 and 359.
Unique study design or methodology (if any): none

Results:

Mortality: no deaths

Clinical signs: no signs with DHEA alone, estrogen related signs seen in monkeys receiving both DHEA and estradiol

Body weights: slight decrease with estradiol and DHEA

Food consumption: no changes

Ophthalmoscopy: no adverse effects

EKG: not done

Hematology: no drug related changes in females and only in males that received E2 with DHEA

Clinical chemistry: no changes with DHEA alone

Urinalysis: no changes

Gross pathology: no DHEA only effects

Organ weights (specify organs weighed if not in histopath table):
Changes in endocrine organ wts (ovary, uterus, vagina, pituitary in females and epididymis, testis, spleen and thymus in males) all attributed to estrogenic effects.

Thyroid weight is also increased although not mentioned by sponsor. Presumably, the weight increase is the result of estrogenic stimulation of SHBG and possible TBG resulting in reduced feedback and increased TSH.

Table 36 Mean (CV) Endocrine/Reproductive Organ Weights in Female Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Organ	Unit	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day)	
					EM-652·HCl (mg/kg/day)	
			2	10	0	40
Adrenals	mg	563	659	584	628	656
		(25)	(29)	(30)	(48)	(38)
	mg/kg BW	102	124	106	123	127
		(7)	(42)	(5)	(21)	(23)
Ovaries	mg	509	688	525	1476	3429
		(22)	(25)	(24)	(164)	(60)
	mg/kg BW	97	124	108	235	707
		(36)	(21)	(51)	(146)	(77)
Pituitary	mg	65.6	65.9	74.7	93.7	67.3
		(24)	(21)	(23)	(26)	(24)
	mg/kg BW	12.1	12.2	14.0	19.5	13.2
		(22)	(29)	(21)	(25)	(15)
Thyroid/ Parathyroids	mg	365	665	569	505	820
		(37)	(24)	(50)	(42)	(52)
	mg/kg BW	65	119	99	100	152
		(17)	(11)	(20)	(31)	(31)
Uterus	g	9.9	12.3	11.5	16.5	4.0
		(39)	(31)	(20)	(46)	(7)
	g/kg BW	1.8	2.2	2.2	3.3	0.8
		(31)	(32)	(31)	(28)	(23)
Vagina	g	8.3	8.3	7.7	9.5	4.0
		(16)	(34)	(20)	(25)	(25)
	g/kg BW	1.6	1.5	1.5	2.0	0.8
		(17)	(3)	(37)	(24)	(40)

Table 37 Mean (CV) Endocrine/Reproductive Organ Weights in Male Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Organ	Unit	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			2	10	0	40
Adrenals	mg	774	807	816	631	799
		(13)	(3)	(27)	(26)	(22)
	mg/kg BW	114	116	122	134	104
		(8)	(17)	(30)	(11)	(7)
Epididymides	g	6.0	6.6	6.0	2.9	6.7
		(16)	(20)	(28)	(75)	(26)
	g/kg BW	0.9	0.9	0.9	0.7	0.9
		(15)	(5)	(24)	(81)	(14)
Pituitary	mg	79.9	87.3	74.6	84.4	57.5
		(23)	(14)	(33)	(21)	(22)
	mg/kg BW	12.0	12.5	10.9	18.4	7.8
		(27)	(14)	(26)	(25)	(30)
Prostate	g	1.8	2.0	1.7	1.3	2.9
		(24)	(13)	(20)	(21)	(22)
	g/kg BW	0.3	0.3	0.3	0.3	0.4
		(18)	(18)	(23)	(35)	(29)
Seminal Vesicles	g	8.2	11.0	9.8	4.0	21.9
		(47)	(36)	(41)	(55)	(77)
	g/kg BW	1.2	1.6	1.5	0.8	2.8
		(37)	(28)	(38)	(57)	(69)
Testes	g	42.0	43.5	38.6	4.2	56.3
		(10)	(35)	(33)	(23)	(26)
	g/kg BW	6.2	6.0	5.7	0.9	7.3
		(9)	(22)	(31)	(11)	(14)
Thyroid/ Parathyroids	mg	648	775	543	294	681
		(35)	(53)	(48)	(13)	(27)
	mg/kg BW	93	106	83	63	93
		(20)	(42)	(53)	(4)	(37)

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (), no (x)

Essentially no histopath changes with DHEA alone.

Table 42 Test Article-Related Histopathologic Findings in Female Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Organ	Finding	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			2	10	0	40
Mammary Gland	Atrophy	1/4	1/4	0/4	0/4	3/4
Ovary	Cyst	0/4	0/4	2/4	0/4	4/4
	Absence of Corpora Lutea	0/4	0/4	1/4	4/4	0/4
Oviduct	Atrophy	0/4	0/4	0/4	0/4	4/4
	Dilatation: Tubule	1/4	1/4	1/4	4/4	0/4
	Hyperplasia/ Hypertrophy	0/4	0/4	0/4	4/4	0/4
	Adenoma	0/4	0/4	0/4	1/4	0/4
Pituitary	Hyperplasia/ Hypertrophy: Acidophils	0/4	0/4	0/4	4/4	0/4
Uterus	Atrophy	0/4	0/4	0/4	0/4	4/4
	Hyperplasia: Endometrial	0/4	0/4	0/4	1/4	0/4
	Inflammation: Perivascular	0/4	0/4	0/4	2/4	0/4
	Metaplasia: Squamous	1/4	2/4	0/4	3/4	0/4
	Dilatation: Endocervical Glands	1/4	0/4	0/4	4/4	0/4
	Degeneration/ Necrosis: Endocervical Glands	0/4	0/4	0/4	2/4	0/4
Vagina	Atrophy	0/4	0/4	0/4	0/4	4/4

Table 43 Test Article-Related Histopathologic Findings in Male Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Organ	Finding	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			2	10	0	40
Epididymis	Atrophy	0/4	0/4	0/4	4/4	0/4
Mammary Gland	Hyperplasia: Duct and/or Alveoli	0/4	0/4	0/4	4/4	0/4
	Ectasia: Duct and/or Alveoli	1/4	2/4	1/4	1/4	4/4
Pituitary	Hyperplasia/ Hypertrophy: Acidophils	0/4	0/4	0/4	4/4	0/4
Prostate	Atrophy	0/4	0/4	0/4	4/4	0/4
	Metaplasia: Squamous	0/4	0/4	0/4	4/4	0/4
Seminal Vesicle	Atrophy	0/4	0/4	1/4	4/4	0/4
Skin/Subcutis	Hyperplasia: Epidermal (Nipple)	0/4	0/4	0/4	4/4	0/4
	Edema (Perineal)	0/4	0/4	0/4	2/4	0/4
Spleen	Atrophy: Lymphoid	0/4	0/4	0/4	3/4	1/4
Testis	Atrophy	1/4	0/4	0/4	4/4	1/4
Thymus	Atrophy: Lymphoid	0/4	0/4	0/4	3/4	2/4

Table 2 Mean (CV) Serum Dehydroepiandrosterone AUC(0-24 hr) Values in Female Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day) EM-652·HCl (mg/kg/day)	
			2	10	0	40
AUC(0-24 hr) (ng·hr/mL)	Day 1	149 (41)	228 (12)	353 (26)	316 (50)	476 (52)
	Day 184	171 (37)	281 (10)	418 (22)	302 (66)	517 (70)
	Day 359	143 (21)	287 (31)	442 (17)	289 (62)	493 (62)

Table 3 Mean (CV) Serum Dehydroepiandrosterone AUC(0-24 hr) Values in Male Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day)	
					EM-652·HCl (mg/kg/day)	
			2	10	0	40
AUC(0-24 hr) (ng·hr/mL)	Day 1	336 (61)	364 (28)	672 (36)	481 (17)	564 (54)
	Day 184	247 (49)	392 (32)	684 (34)	853 (132)	425 (67)
	Day 359	236 (38)	313 (37)	586 (41)	317 (33)	455 (60)

Table 4 Mean (CV) Serum Dehydroepiandrosterone Sulfate AUC(0-24 hr) Values in Female Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day)	
					EM-652·HCl (mg/kg/day)	
			2	10	0	40
AUC(0-24 hr) (μg·hr/mL)	Day 1	2.19 (63)	18.9 (36)	47.1 (47)	49.0 (39)	53.7 (24)
	Day 184	1.81 (61)	15.9 (34)	42.3 (27)	43.4 (46)	51.2 (31)
	Day 359	1.48 (45)	18.1 (45)	45.7 (34)	50.7 (45)	64.8 (28)

Table 5 Mean (CV) Serum Dehydroepiandrosterone Sulfate AUC(0-24 hr) Values in Male Monkeys Following Oral Administration of Dehydroepiandrosterone, EM-652·HCl and Estradiol

Parameter (Unit)	Interval	Control	DHEA (mg/kg/day)		DHEA (10 mg/kg/day) and Estradiol (0.3 mg/kg/day)	
					EM-652·HCl (mg/kg/day)	
			2	10	0	40
AUC(0-24 hr) (μg·hr/mL)	Day 1	2.78 (66)	19.3 (27)	50.9 (39)	43.6 (10)	57.3 (17)
	Day 184	2.14 (57)	15.0 (26)	47.7 (34)	43.6 (22)	72.5 (14)
	Day 359	1.78 (56)	15.2 (18)	46.9 (21)	39.4 (19)	79.5 (26)

Female monkeys given 2 and 10 mg/kg DHEA orally for 184 days had serum AUC's$_{0\text{-}24h}$ of 281 and 418 ng.h/ml and males had AUC's of 392 and 684 ng.h/ml. Women given 0.5% (6.5 mg) DHEA intravaginally for 7 days had a mean serum AUC$_{0\text{-}24h}$ of 56 ng.h/ml. Exposure margins are 5 and 7X for females and 7 and 12X for males.

NOAEL is 10 mg/kg assuming the effects on organ weights are pharmacologic.

7 Genetic Toxicology

Study title: Salmonella-Escherichia/Mammalian-Microsome reverse mutation assay

Key findings: Study was negative.

Study no.: 7220-105

Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: march, 2004
GLP compliance: yes
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: EM-760, batch no. EM-760-17

Methods

Strains/species/cell line:

Table I. Tester Strain Genotypes

Tester Strain	*his*/*trp* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA97a	*his*D6610	*uvr*B	*rfa*	pKM101
TA102	*his*G428	-	*rfa*	pKM101/pAQ1
WP2*uvr*A	*trp*	*uvr*A	–	–

Doses used in definitive study:
Doses for all strains were 66, 250, 660, 1380, 2250, and 5000 ug/plate in the presence or absence of S9 mix.

Basis of dose selection:
Dose ranging study; maximum dose

Negative controls:
Vehicle

Positive controls:

Table II. Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA97a	+	2-aminoanthracene	2.5
TA97a	–	ICR-191	2.0
TA102	+	2-aminoanthracene	15.0
TA102	–	mitomycin C	1.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times:
Bacteria were cultured in agar on plates for 52 hrs.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Study included three trials with replicates with each trial. Positive control had to have 3 fold increase over vehicle control. Study was valid.

Study outcome:

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[b]
	Dose/Plate	TA100		TA1535		TA97a		TA102		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		115	29	17	5	202	9	420	17	11	2	N
Test Article	66.0 µg	105	6	13	3	191	9	485	13	16	5	N
	250 µg	111	7	19	4	178	10	500	28	17	4	N
	660 µg	107	4	15	4	183	6	458	80	17	3	N
	1380 µg	88	8	13	3	169	2	411	4	17	2	NP
	2250 µg	86	2	9	2	141	21	349	14	17	5	NP
	5000 µg	71	5	11	5	167	25	243	19	9	6	NP/OP[e]
Positive Control[c]		1211	74	115	7	1301	84	3193	132	186	6	N
Microsomes: None												
Vehicle Control		102	16	15	1	132	14	306	16	15	4	N
Test Article	66.0 µg	85	6	13	3	111	9	238	27	22	4	N
	250 µg	77	11	15	3	100	7	232	41	16	1	N
	660 µg	68	2	11	3	97	13	247	29	18	6	N
	1380 µg	44	18	7	1	72	15	167	19	11	1	NP/RP[e]
	2250 µg	62	8	8	2	79	17	74	46	8	2	NP/RP[e]
	5000 µg	43	4	6	3	46	11	31	19	6	2	NP/RP[e]
Positive Control[d]		1120	30	700	151	3234	184	2252	159	485	80	N

Study was negative with or without metabolic activation

Study title: Chromosome aberrations of EM-760 in human peripheral blood lymphocytes

Key findings: Study was negative

Study no.: 7220-106

Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: March, 2004
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: EM-760, batch no. EM-760-17

Methods

Strains/species/cell line:
Human peripheral venous whole blood with and without S9.
Lymphocytes normally do not divide, but they were stimulated to divide in cultures by exposure to phytohemagglutinin (PHA). At predetermined intervals after exposure to the test article, the lymphocytes were treated with a metaphase arresting substance, Colcemid, harvested, stained, and metaphase cells were analyzed for the presence of chromosomal aberrations.

Doses used in definitive study:
-S9 (4hr exposure): 400, 300, 225, 200, 175,……50 ug/ml
-S9 (19 hr exposure): 138, 100, 66, 50……..6.6 ug/ml
+S9 (4 hr exposure): 400, 300……66 ug/ml

Basis of dose selection:
High dose exceeded the solubility limit of the test system

Negative controls:
1 % ethanol vehicle

Positive controls:
mitomycin C (MMC) for nonactivation series; cyclophosphamide (CP) in the metabolic activation series.

Incubation and sampling times: See above.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Duplicates in each test. The negative and vehicle control cultures must contain less than 5% cells with aberrations. Positive control must be significantly higher (P<0.01) that vehicle controls. Study was valid

Study outcome:

Table 3: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 4-Hour Treatment, ~22-Hour Harvest

Assay No.: 25725-0-449OECD1 Trial No.: B1 Date: 04/07/04 Lab No.: CY041304 Test Article: EM-760

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	gaps	simple breaks	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: chte	chre	mab	Totals[c] g-	Totals[c] g+	Judgement (+/-)[d]
Controls																	
Negative:	RPMI 1640		A	100		100	0	0							0	0	
			B	100		100	0	0		2	1				1	3	
			Total	**200**		**200**				2	1				1	3	
			Average	**%**	--		**0.0**	**0.0**		**1.0**	**0.5**				**0.5**	**1.5**	
Vehicle:	Ethanol	10.0 µL/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		3					0	3	
			Total	**200**		**200**				6	1				1	7	
			Average	**%**	**0**		**0.0**	**0.0**		**3.0**	**0.5**				**0.5**	**3.5**	
Positive:	MMC	1.00 µg/mL	A	75		100	0	0		7	11	8		1	18	25	
			B	75		100	0	0		10	12	6			15	24	
			Total	**150**		**200**				17	23	14		1	33	49	
			Average	**%**	**33**		**0.0**	**0.0**	-	**11.3**	**15.3**	**9.3**		**0.7**	**22.0**	**32.7**	+
Test Article		138 µg/mL	A	100		100	0	0		2	1				1	3	
			B	100		100	0	0		6					0	6	
			Total	**200**		**200**				8	1				1	9	
			Average	**%**	**19**		**0.0**	**0.0**	-	**4.0**	**0.5**				**0.5**	**4.5**	-
		175 µg/mL	A	100		100	0	0		3	3				3	6	
			B	100		100	0	0		5					0	5	
			Total	**200**		**200**				8	3				3	11	
			Average	**%**	**25**		**0.0**	**0.0**	-	**4.0**	**1.5**				**1.5**	**5.5**	-
		200 µg/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		3	1				1	4	
			Total	**200**		**200**				6	2				2	8	
			Average	**%**	**41**		**0.0**	**0.0**	-	**3.0**	**1.0**				**1.0**	**4.0**	-
		225 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		6					0	6	
			Total	**200**		**200**				8					0	8	
			Average	**%**	**47**		**0.0**	**0.0**	-	**4.0**					**0.0**	**4.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium MMC = Mitomycin C

Table 5: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~19-Hour Treatment, ~22-Hour Harvest

Assay No.: 25725-0-449OECD1 Trial No.: B1 Date: 04/07/04 Lab No.: CY041304 Test Article: EM-760

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] g-	Totals[c] g+	Judgement (+/-)[d]
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		1					0	1	
			B	100		100	0	0		2	1				1	3	
			Total	**200**		**200**				3	1				1	4	
			Average	**%**	--		**0.0**	**0.0**		**1.5**	**0.5**				**0.5**	**2.0**	
Vehicle:	Ethanol	10.0 µL/mL	A	100		100	0	0							0	0	
			B	100		100	0	0		3	1				1	4	
			Total	**200**		**200**				3	1				1	4	
			Average	**%**	**0**		**0.0**	**0.0**		**1.5**	**0.5**				**0.5**	**2.0**	
Positive:	MMC	0.300 µg/mL	A	50		100	0	0		2	18	7			23	24	
			B	50		100	0	0		2	21	1			22	23	
			Total	**100**		**200**				4	39	8			45	47	
			Average	**%**	**58**		**0.0**	**0.0**	-	**4.0**	**39.0**	**8.0**			**45.0**	**47.0**	+
Test Article		25.0 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	**200**		**200**				3					0	3	
			Average	**%**	**24**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		50.0 µg/mL	A	143		100	0	0		1	1				1	2	
			B	57		100	0	0		1		1			1	2	
			Total	**200**		**200**				2	1	1			2	4	
			Average	**%**	**3**		**0.0**	**0.0**	-	**1.0**	**0.5**	**0.5**			**1.0**	**2.0**	-
		66.0 µg/mL	A	100		100	0	0		4	1				1	5	
			B	100		100	0	0		1	1				1	2	
			Total	**200**		**200**				5	2				2	7	
			Average	**%**	**0**		**0.0**	**0.0**	-	**2.5**	**1.0**				**1.0**	**3.5**	-
		100 µg/mL	A	100		100	0	0		5	3				3	7	
			B	100		100	0	0		7	2				2	8	
			Total	**200**		**200**				12	5				5	15	
			Average	**%**	**49**		**0.0**	**0.0**	-	**6.0**	**2.5**				**2.5**	**7.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium MMC = Mitomycin C

Table 7: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 4-Hour Treatment, ~22-Hour Harvest

Assay No.: 25725-0-449OECD1 Trial No.: B1 Date: 04/07/04 Lab No.: CY041304 Test Article: EM-760

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] g-	Totals[c] g+	Judgement (+/-)[d]
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		1					0	1	
			B	100		100	0	0							0	0	
			Total	**200**		**200**				1					0	1	
			Average	**%**	--		**0.0**	**0.0**		**0.5**					**0.0**	**0.5**	
Vehicle:	Ethanol	10.0 µL/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0		1	2				2	3	
			Total	**200**		**200**				2	3				3	5	
			Average	**%**	**0**		**0.0**	**0.0**		**1.0**	**1.5**				**1.5**	**2.5**	
Positive:	CP	25.0 µg/mL	A	50		100	0	0		15	16	2			17	27	
			B	75		100	0	0		19	15	2			17	34	
			Total	**125**		**200**				34	31	4			34	61	
			Average	**%**	**50**		**0.0**	**0.0**	-	**27.2**	**24.8**	**3.2**			**27.2**	**48.8**	+
Test Article		138 µg/mL	A	100		100	0	0		6					0	6	
			B	100		100	0	0		6					0	6	
			Total	**200**		**200**				12					0	12	
			Average	**%**	**9**		**0.0**	**0.0**	-	**6.0**					**0.0**	**6.0**	-
		175 µg/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		3					0	3	
			Total	**200**		**200**				6	1				1	7	
			Average	**%**	**14**		**0.0**	**0.0**	-	**3.0**	**0.5**				**0.5**	**3.5**	-
		225 µg/mL	A	100		100	0	0		2	3				3	5	
			B	100		100	1	0		9		1			1	10	
			Total	**200**		**200**				11	3	1			4	15	
			Average	**%**	**9**		**0.5**	**0.0**	-	**5.5**	**1.5**	**0.5**			**2.0**	**7.5**	-
		300 µg/mL	A	129		100	0	0		8	4				4	12	
			B	79		100	0	0		11	6				6	13	
			Total	**208**		**200**				19	10				10	25	
			Average	**%**	**70**		**0.0**	**0.0**	-	**9.1**	**4.8**				**4.8**	**12.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium CP = Cyclophosphamide

Study was negative with and without metabolic activation

Study title: In Vivo mouse bone marrow micronucleus assay

Key findings: study was negative

Study no.: 7220-107

Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: April, 2006
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: EM-760, lot no. 2004OCT0401, 100% pure

Methods

Strains/species/cell line: CD-1 mice

Doses used in definitive study:

Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/Harvest Timepoint 24 Hour Male	48 Hour Male
Positive Control, 80	8	10	Oral Gavage	5	-
Vehicle Control, 0	0	10	Oral Gavage	5	5
500	50	10	Oral Gavage	5	-
1000	100	10	Oral Gavage	5	-
2000	200	10	Oral Gavage	5	5

Vehicle Control = 0.4% methylcellulose, Positive Control = Cyclophosphamide

Basis of dose selection:
Dose range finding study

Negative controls:
methylcellulose

Positive controls:
Cyclophosphomide (CP)

Incubation and sampling times:
24 and 48 hrs

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): vehicle control should be less than 0.4% micronucleated PCE's. Statistically significant elevation of the positive control gp relative to the vehicle control gp. Study was valid.

Study outcome:

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	0.4% MC 10 mL/kg	24 hr	0.02 ± 0.01	0.72 ± 0.03
		48 hr	0.04 ± 0.01	0.48 ± 0.05
Positive	CP 80 mg/kg	24 hr	3.36 ± 0.34*	0.38 ± 0.04**
Test Article	500 mg/kg	24 hr	0.02 ± 0.01	0.71 ± 0.09
	1000 mg/kg	24 hr	0.01 ± 0.01	0.79 ± 0.07
	2000 mg/kg	24 hr	0.02 ± 0.01	0.71 ± 0.10
		48 hr	0.03 ± 0.01	0.56 ± 0.10

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
MC = Methylcellulose
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Study was negative.

8 Carcinogenicity

None conducted.

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9 Reproductive and Developmental Toxicology

None conducted.

10 Special Toxicology Studies

None conducted.

11 Integrated Summary and Safety Evaluation

Endoceutics intends to market Intrarosa (prasterone) for treatment of dyspareunia due to vulvovaginal atrophy in postmenopausal women.

Prasterone (DHEA) and its sulfated metabolite (DHEA-S) are the most abundant steroids in the body. DHEA is synthesized in the adrenals and is the biosynthetic precursor to sex hormones.

Vaginal and percutaneous administration of DHEA prevents the decreased weight and histological signs of vaginal atrophy in ovariectomized rats.

In 6 month rat and 12 month monkey oral toxicology studies, DHEA was essentially non-toxic and produced no adverse effects in female monkeys at dose up to 10 mg/kg (7X human exposure by AUC). In female rats, there were some estrogen/androgen related effects including minimal to slight squamous metaplasia of the glandular epithelium of the uterus at doses of 10 to 100 mg/kg (3 to 16X human exposure).

DHEA was negative in three genotoxicity studies: bacterial mutagenesis assay, in vitro chromosomal aberrations assay with human peripheral blood lymphocytes, and in vivo mouse bone marrow micronucleus assay.

No carcinogenicity studies were conducted and none are required. DHEA is a non-genotoxic endogenous hormone given in a small amount (6.5 mg) intravaginally essentially as replacement therapy exclusively for local action. There is an absence of cause for concern based on results of the one year toxicology study in monkeys and a series of clinical studies performed with DHEA vaginal suppositories. There is no biologically significant systemic exposure at the proposed therapeutic dose. There are a large amount of data available in the public domain (DHEA is available in the U.S. without a prescription; 10 to 100 mg capsules and 1% cream can be obtained from health food stores and the internet).

No reproduction studies were submitted and none are required. The indication is for use in postmenopausal women. DHEA is metabolized to androgens and estrogens in the vagina and should be contraindicated in pregnancy (similar to premarin).

In summary, prasterone (DHEA) as well as its sulfated metabolite DHEA-S, are endogenous steroid hormones that circulate at high levels in premenopausal women. Levels decline with age and replacement therapy with DHEA may help reverse postmenopausal vaginal atrophy associated with the reduction in endogenous estrogens. DHEA is metabolized to testosterone and estradiol by intracellular enzymes located within the vaginal cells and very little reaches the systemic circulation. The human TK data show that systemic sex hormone levels in DHEA treated postmenopausal women are essentially the same as in women receiving a placebo. The six-month rat and one year monkey toxicology studies did not reveal any significant adverse effects of DHEA treatment.

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/s/

ALEXANDER W JORDAN
08/09/2016

MUKESH SUMMAN
08/09/2016
Nonclinical supports AP