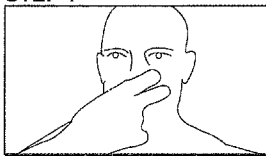




US 20130045958A1

(19) **United States**(12) **Patent Application Publication**
Kreppner et al.(10) **Pub. No.: US 2013/0045958 A1**(43) **Pub. Date: Feb. 21, 2013**(54) **INTRANASAL 0.15% AND 0.24%
TESTOSTERONE GEL FORMULATIONS AND
USE THEREOF FOR TREATING
ANORGASMIA OR HYPOACTIVE SEXUAL
DESIRE DISORDER**filed on May 14, 2011, provisional application No.
61/513,499, filed on Jul. 29, 2011, provisional appli-
cation No. 61/598,335, filed on Feb. 13, 2012.**Publication Classification**(75) Inventors: **Wayne Kreppner**, Georgetown (CA);
Siobhan Fogarty, Blackrock (IE);
Werner Oberegger, Waterdown (CA);
Paul Maes, Vise (BE)(51) **Int. Cl.**
A61K 31/568 (2006.01)
A61P 15/10 (2006.01)(73) Assignee: **Trimel Pharmaceuticals Corporation**,
Mississauga (CA)(52) **U.S. Cl.** **514/178**(21) Appl. No.: **13/471,450**(22) Filed: **May 14, 2012****Related U.S. Application Data**(60) Provisional application No. 61/518,903, filed on May
13, 2011, provisional application No. 61/486,254,(57) **ABSTRACT**

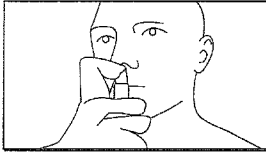
The present invention relates to lower dosage strength perna-
sal testosterone gel formulations for intranasal administration
and treatment methods for using the lower dosage strength
pernasal testosterone gel formulations for treating a female
subject with anorgasmia and/or hypoactive sexual desire dis-
order.

STEP 1

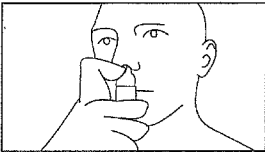
Blow your nose to clear your nostrils.

STEP 2

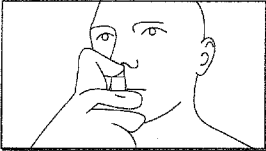
Remove the cap.

STEP 3

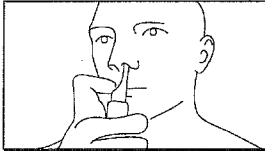
Place your finger on the pump of the actuator
and in front of a mirror, advance the tip of the
actuator into your left nostril until the finger
on the pump reaches the base of the nose.

STEP 4

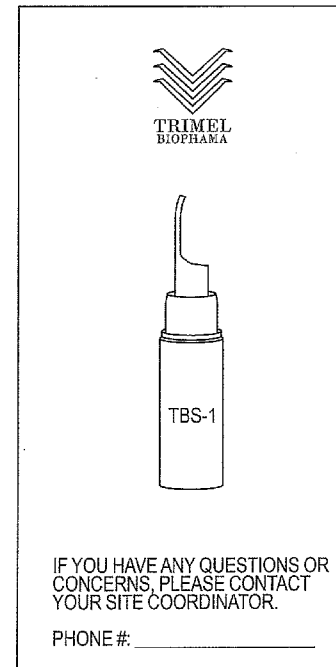
Aim the tip of the actuator to the inner corner
of your left eye. The opening on the tip of the
actuator must face the nasal mucosa.

STEP 5

Depress the pump until it stops.

STEP 6

Slowly remove the actuator from your nose.



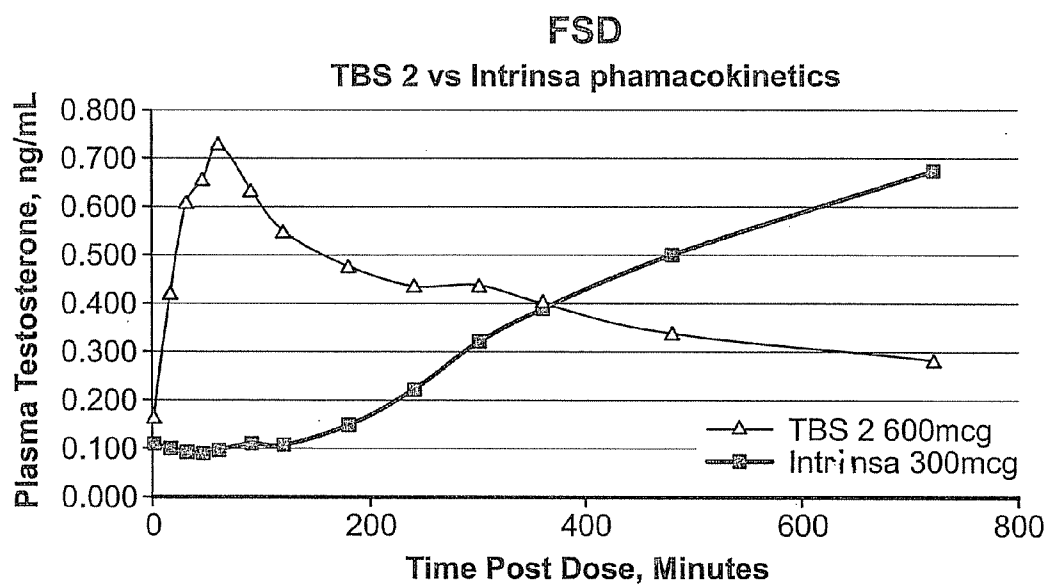


FIG. 1

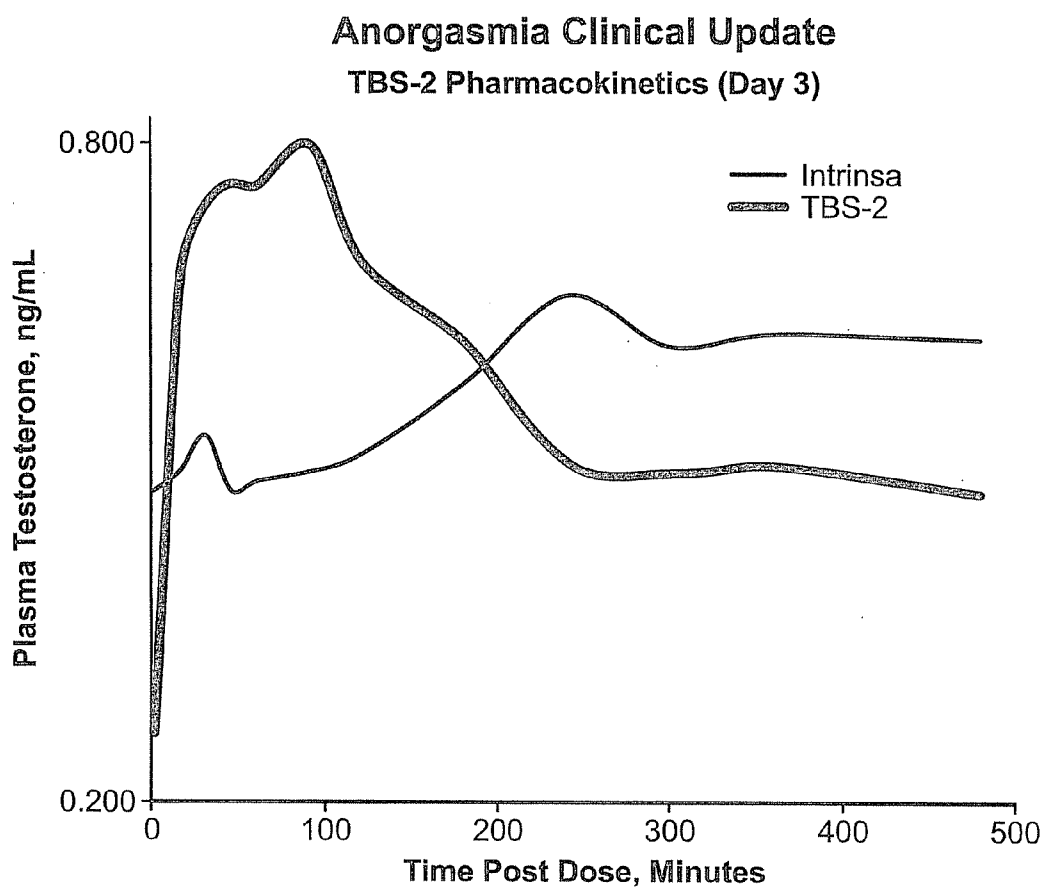


FIG. 2

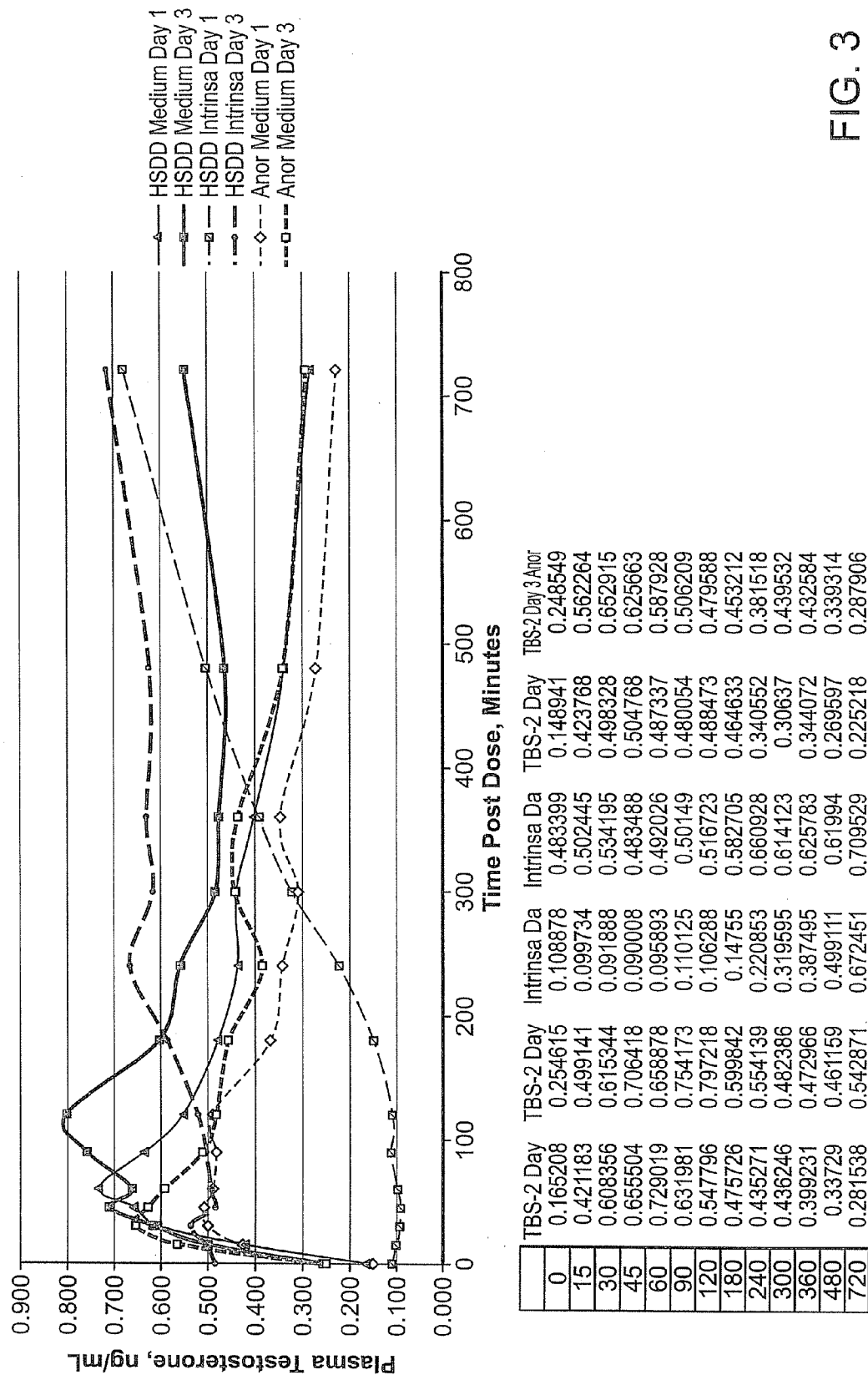


FIG. 3

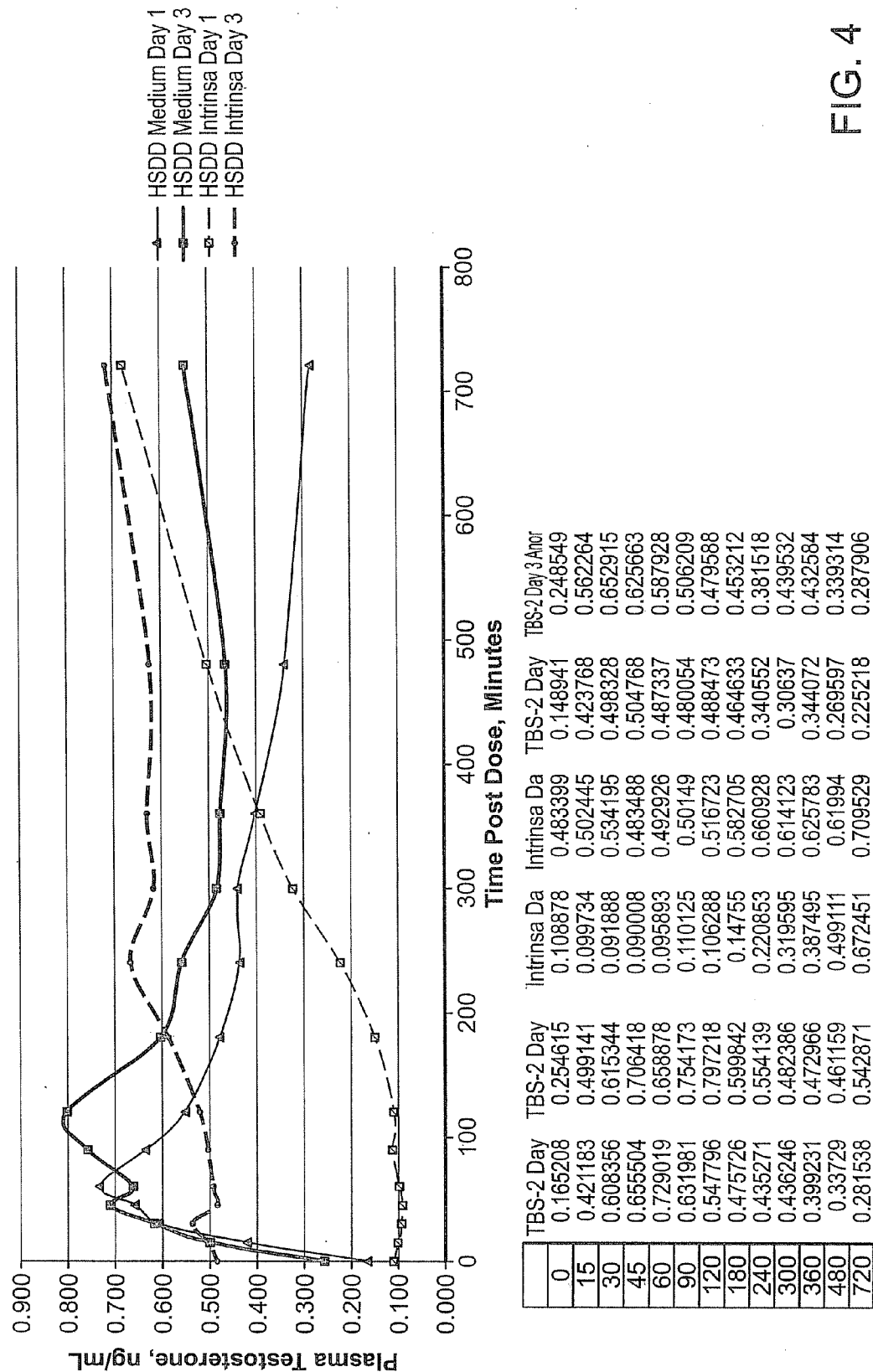


FIG. 4

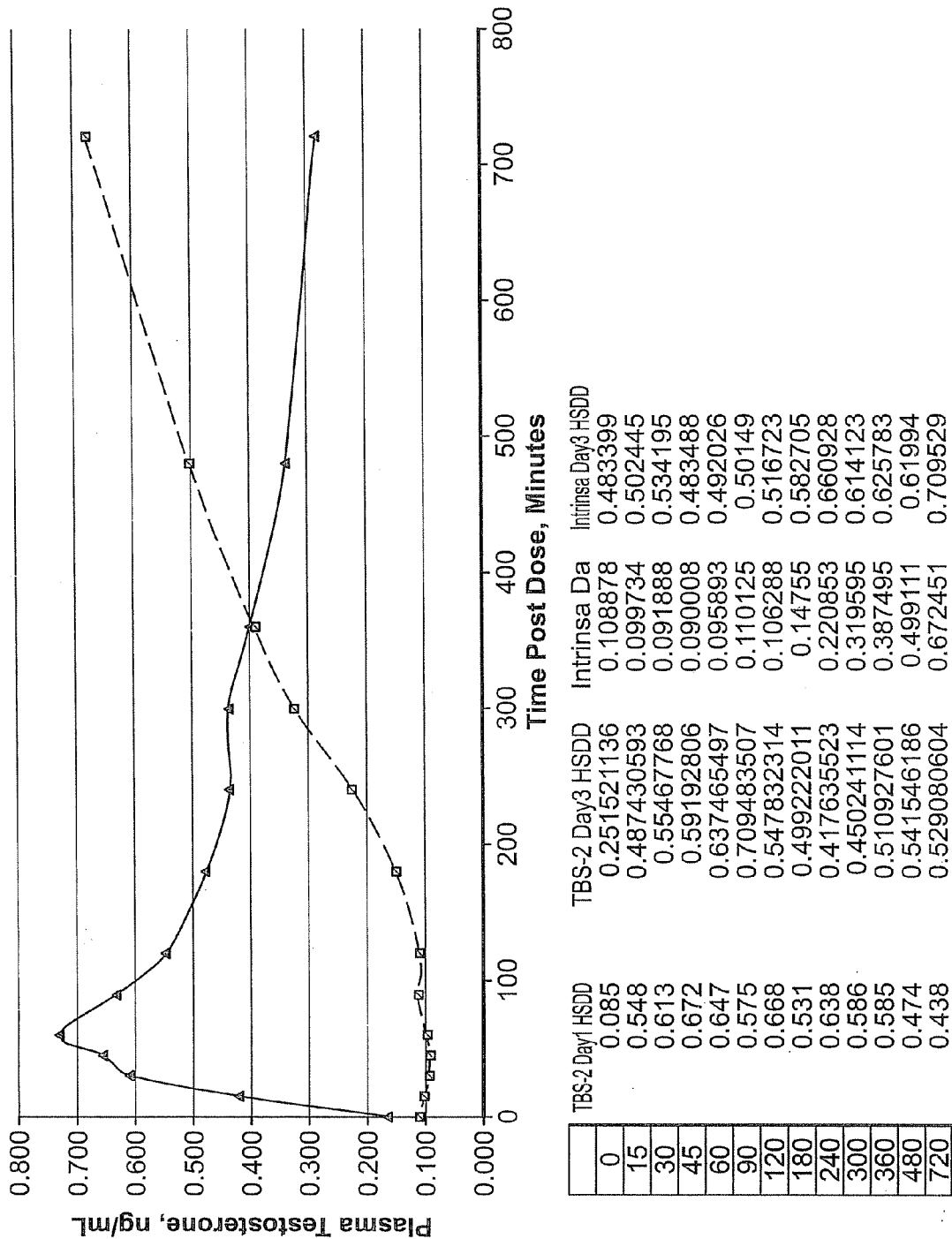


FIG. 5

	TBS-2 Day1 HSDD	TBS-2 Day3 HSDD	Intrinsa Da	Intrinsa Day3 HSDD
0	0.085	0.251521136	0.108878	0.483399
15	0.548	0.487430593	0.099734	0.502445
30	0.613	0.55467768	0.091888	0.534195
45	0.672	0.59192806	0.090008	0.483488
60	0.647	0.637465497	0.095893	0.492026
90	0.575	0.709483507	0.110125	0.50149
120	0.668	0.547832314	0.106288	0.516723
180	0.531	0.499222011	0.14755	0.582705
240	0.638	0.417635523	0.220853	0.660928
300	0.586	0.450241114	0.319595	0.614123
360	0.585	0.510927601	0.387495	0.625783
480	0.474	0.541546186	0.499111	0.61994
720	0.438	0.529080604	0.672451	0.709529

FIG. 6

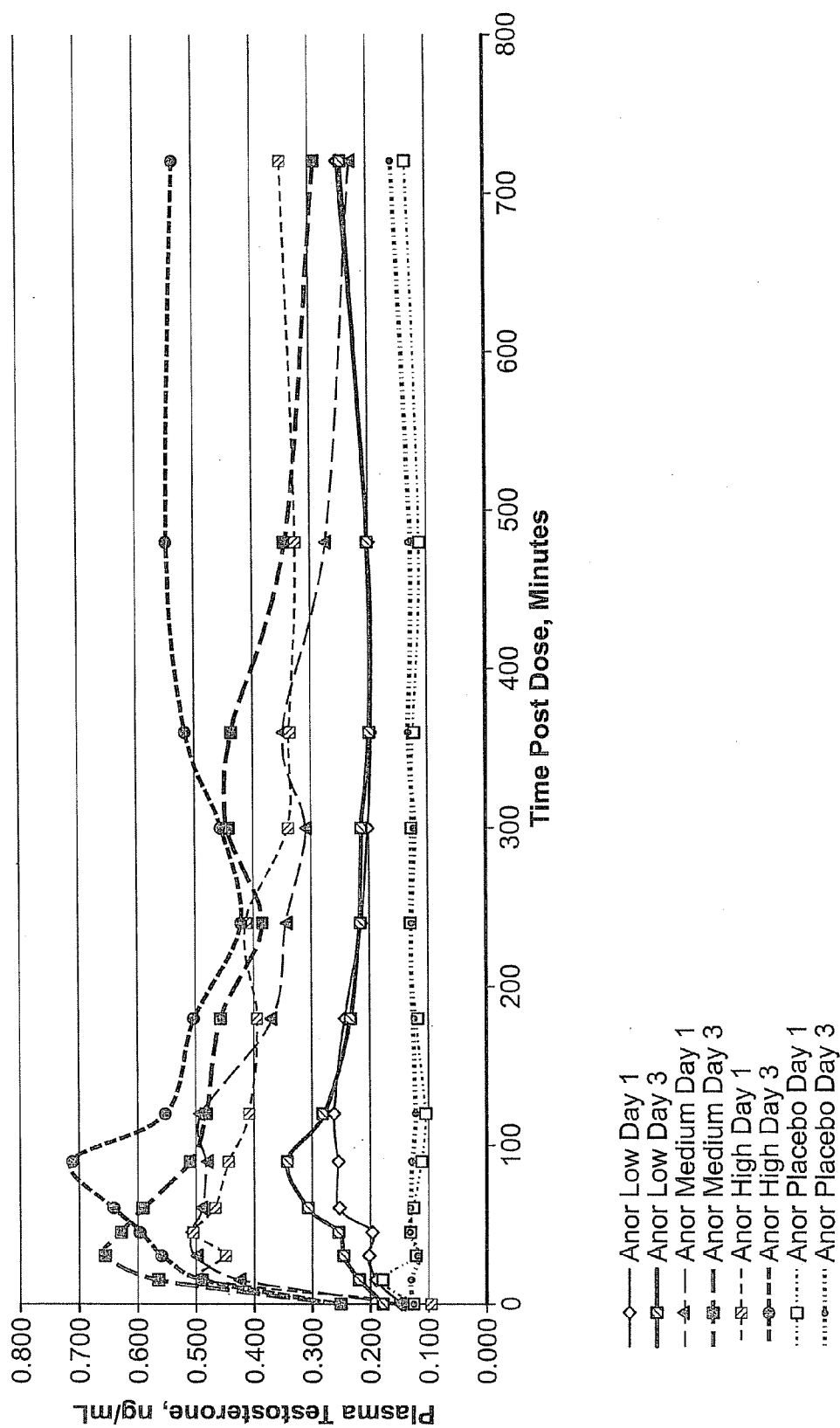


FIG. 7

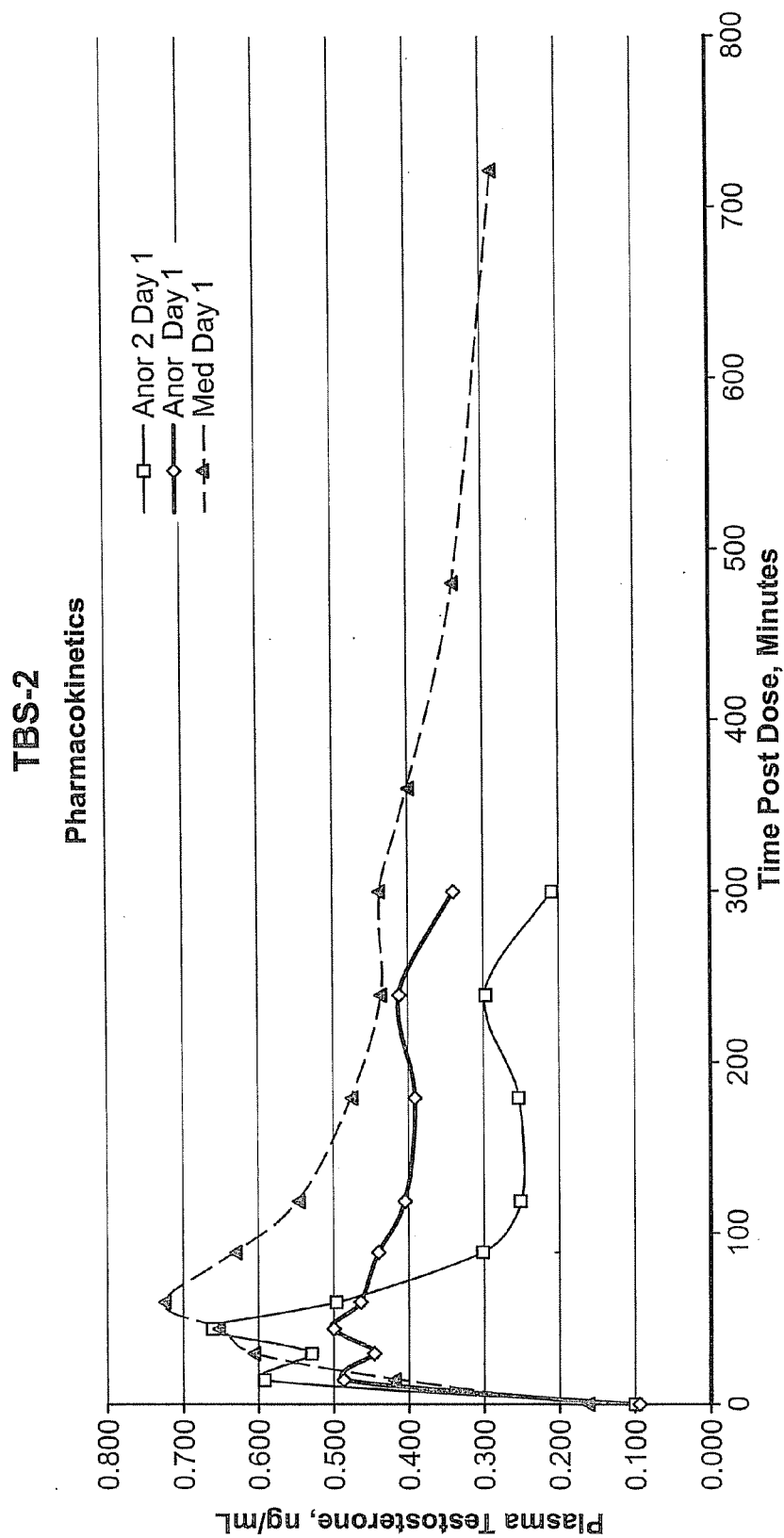


FIG. 8

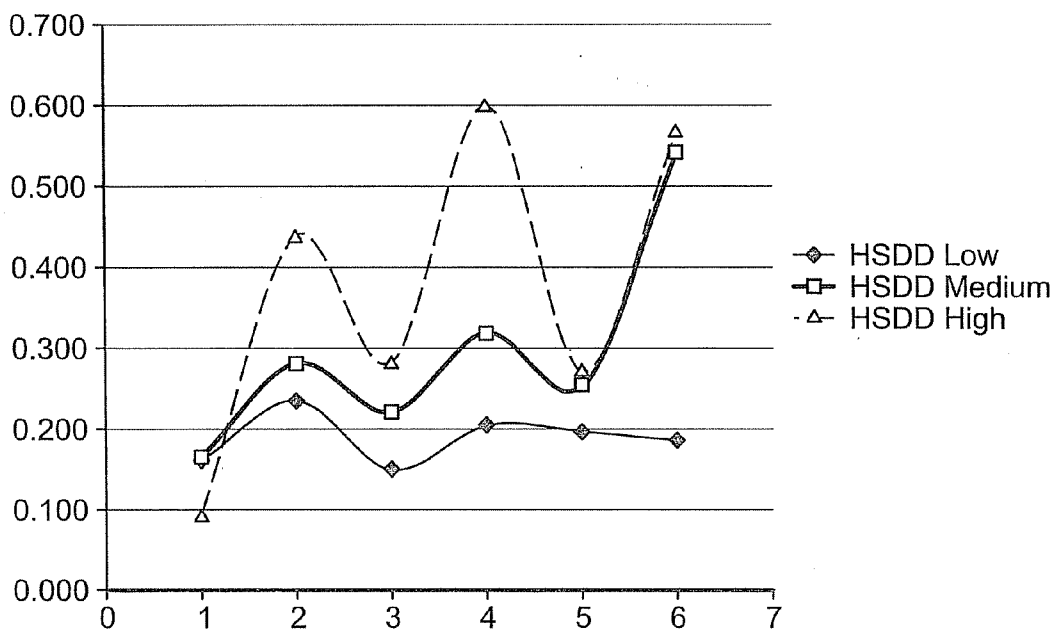
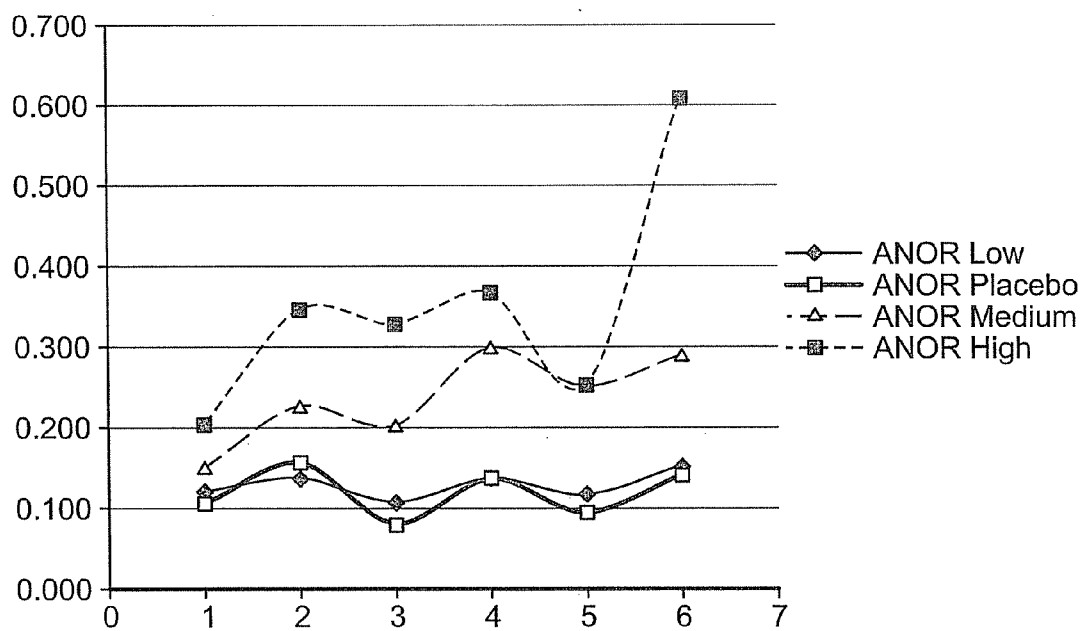


FIG. 9

	Placebo		Low		Medium		High		Intrinsa	
	ANOR	HSDD	ANOR	HSDD	ANOR	HSDD	ANOR	HSDD	ANOR	HSDD
AUC12 Day 1	84.016		154.468	159.337	232.064	290.543	261.208	415.519		265.943
AUC12 Day 3	100.328		182.745	162.478	321.319	434.447	405.019	491.815		479.142
Cavg12 Day 1	7.001		12.872	13.278	19.339	24.212	21.767	34.627		22.162
Cavg12 Day 3	8.361		15.229	13.540	26.777	36.204	33.752	40.985		39.929
Cmax12 Day 1	0.154		0.301	0.304	0.553	0.786	0.727	1.192		0.685
Cmax12 Day 3	0.157		0.356	0.287	0.799	0.937	0.750	1.197		0.712

FIG. 10

ANOR									
Testosterone	Placebo	Placebo	Placebo	Placebo	Low	Low	Low	Low	High
Patient Initials	MT	AN	RS	ISE	JSC	LK	WY	AFO	ER
Subject #	1020	1024	2025	2029	1017	1022	2028	2030	1018
Day 1 2000	0.191	0.0598	0.171	0.105	0.182	0.175	0.164	0.0943	0.666
Day 2 0800	0.18	0.0756	0.163	0.154	0.263	0.213	0.202	0.328	0.332
Day 2 2000	0.158	0.0551	0.187	0.0794	0.167	0.154	0.207	0.13	0.374
Day 3 0800	0.207	0.0693	0.175	0.135	0.192	0.179	na	0.565	0.412
Day 3 2000	0.195	0.0654	0.150	0.0936	0.173	0.178	na	0.169	0.348
Day 4 0800	0.245	0.0734	0.207	0.139	0.240	0.178	na	0.325	0.869
Mean									
	0.202				0.120				0.149
	0.345				0.136				0.225
	0.326				0.107				0.201
	0.365				0.136				0.297
	0.252				0.116				0.249
	0.604				0.151				0.288
HSDD									
Testosterone	Intrinsa	Intrinsa	Intrinsa	Intrinsa	Low	Low	Low	Low	High
Patient Initials	BK	AO	MG	HJ	WN	RK	RST	RKE	DB
Subject #	1004	1006	1104	1106	1002	1007	2010	2009	1008
Day 1 2000	0.0889	0.189	0.112	0.133	0.154	0.268	0.101	0.0912	0.105
Day 2 0800					0.199	0.305	0.216	0.286	0.569
Day 2 2000					0.181	0.168	0.111	0.285	0.424
Day 3 0800					0.227	0.214	0.176	0.402	1.060
Day 3 2000					0.233	0.237	0.141	0.242	0.422
Day 4 0800	0.758	1.02	0.462	0.366	0.186	0.214	0.163	0.219	1.400
Mean									
	0.093				0.161				0.085
	0.438				0.236				0.516
	0.282				0.150				0.186
	0.600				0.204				0.508
	0.273				0.198				0.199
	0.570				0.187				0.603

FIG. 11

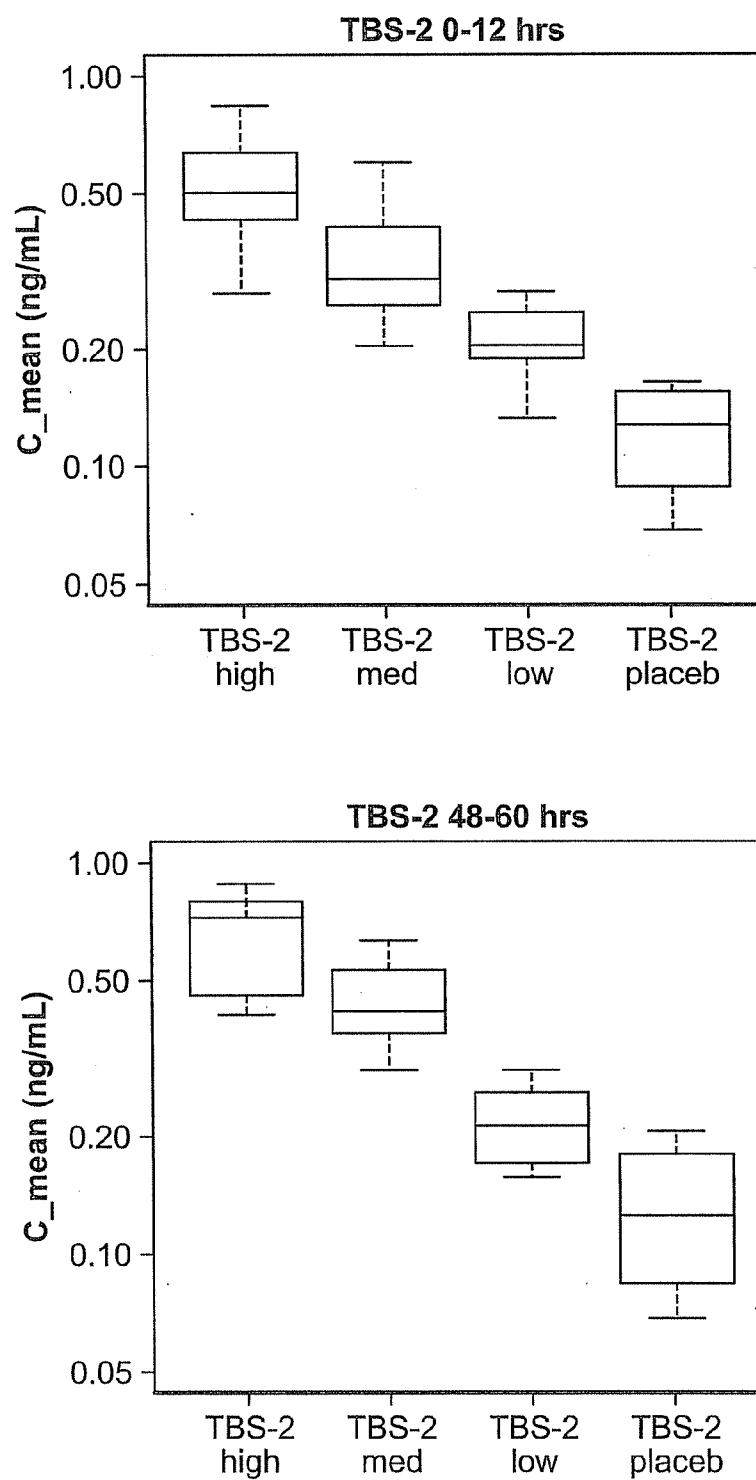


FIG. 12

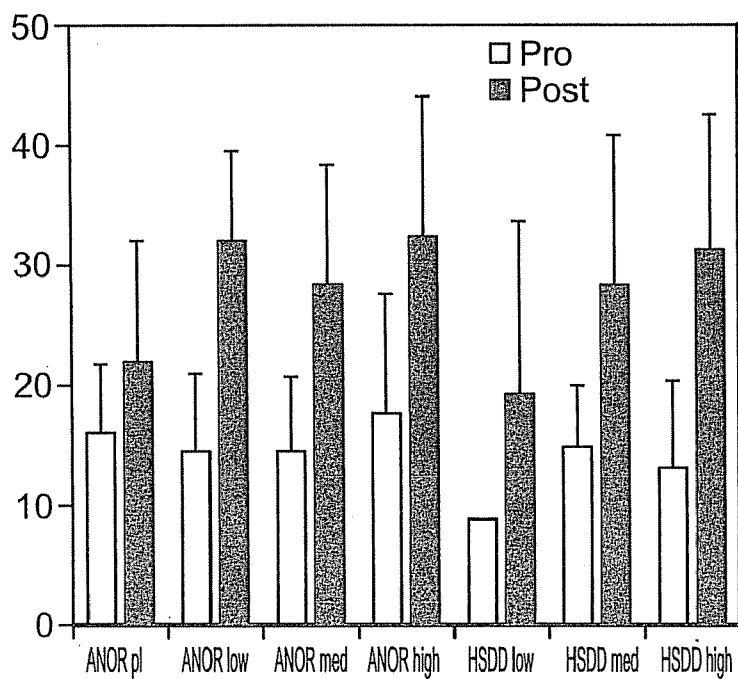
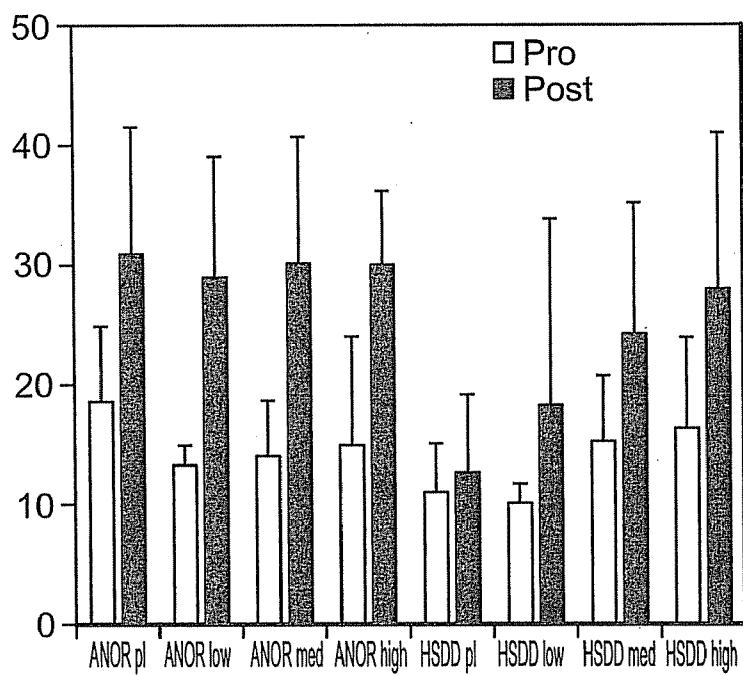


FIG. 13

Mean Testosterone Levels Following TBS-2 or Placebo Administration
(hours 0-12) in Women with Anorgasmia

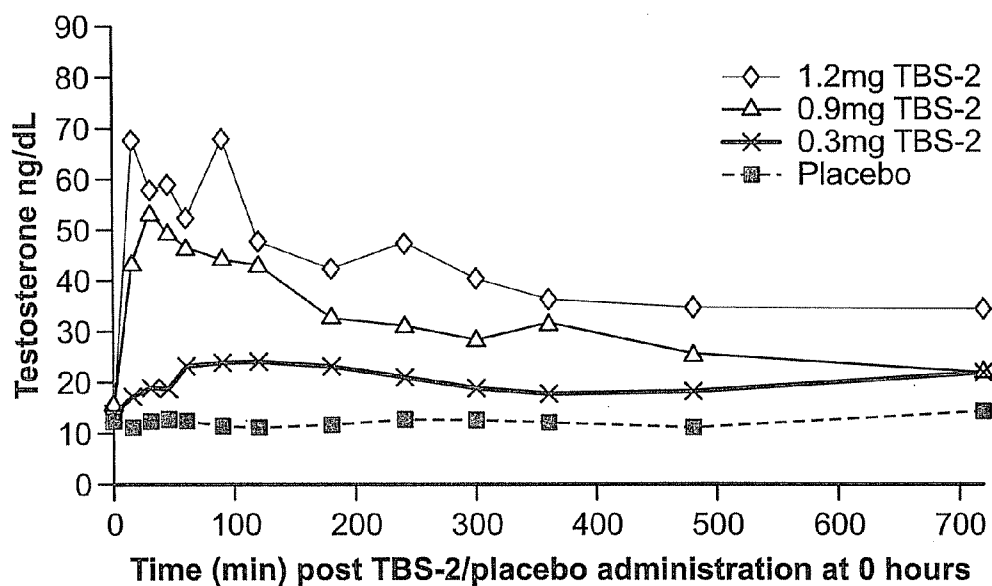


FIG. 14

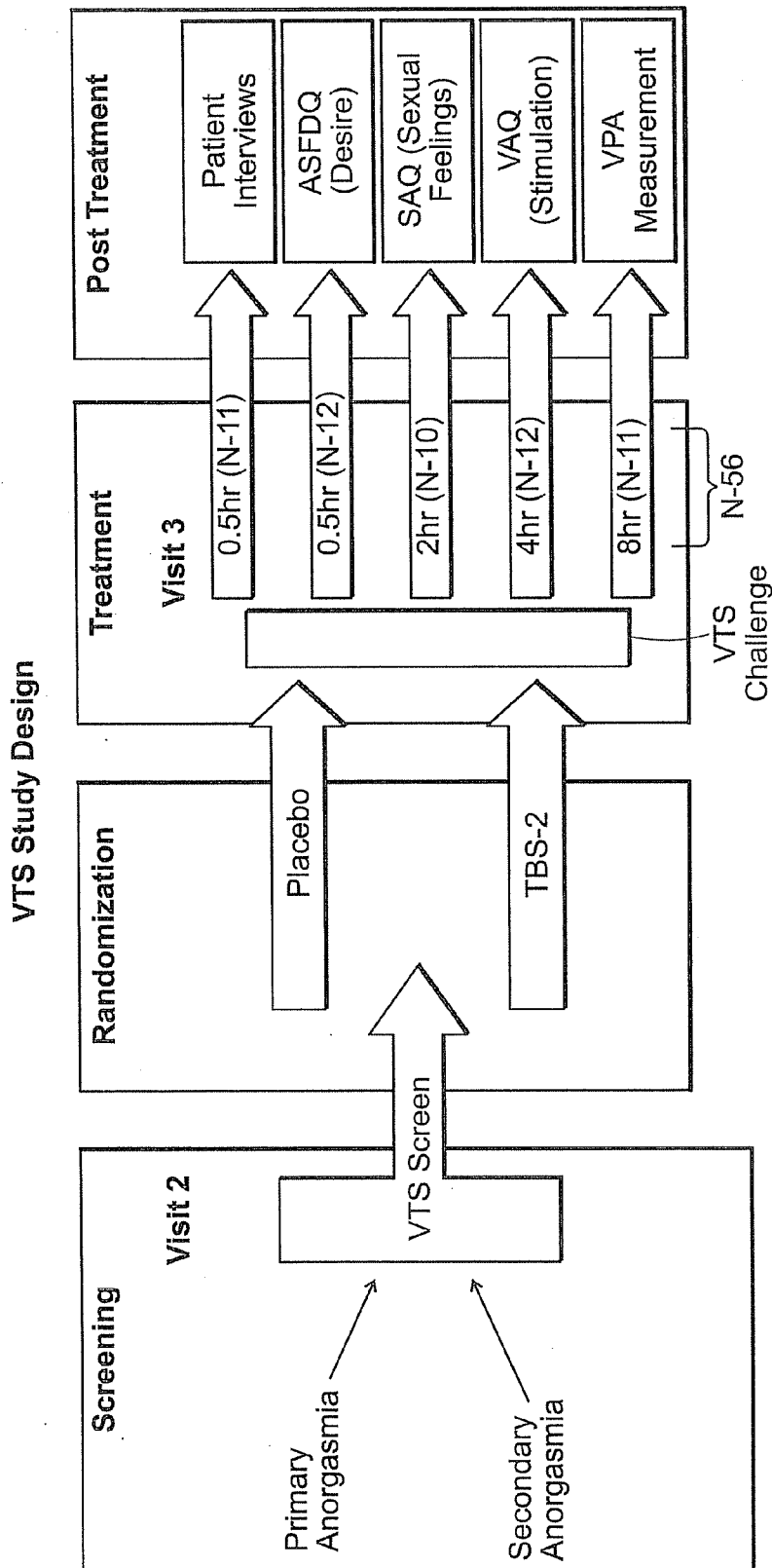


FIG. 15

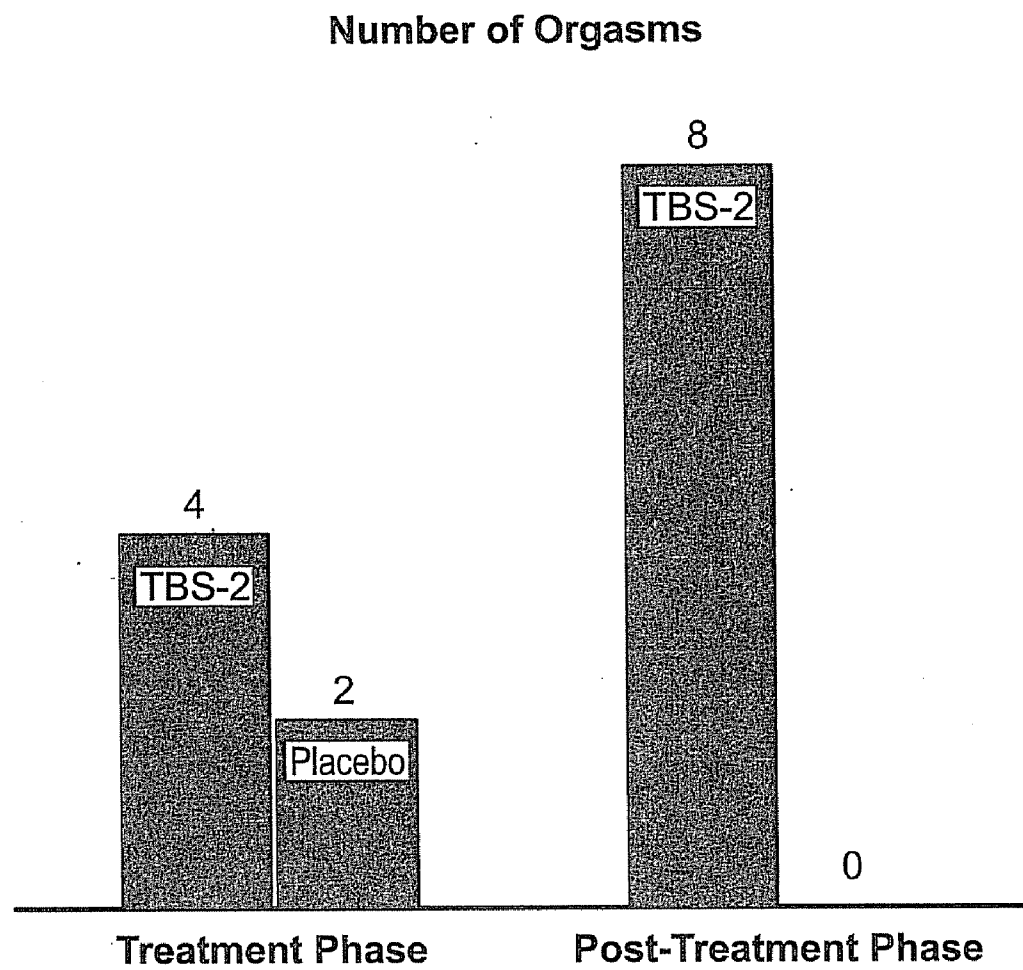


FIG. 16

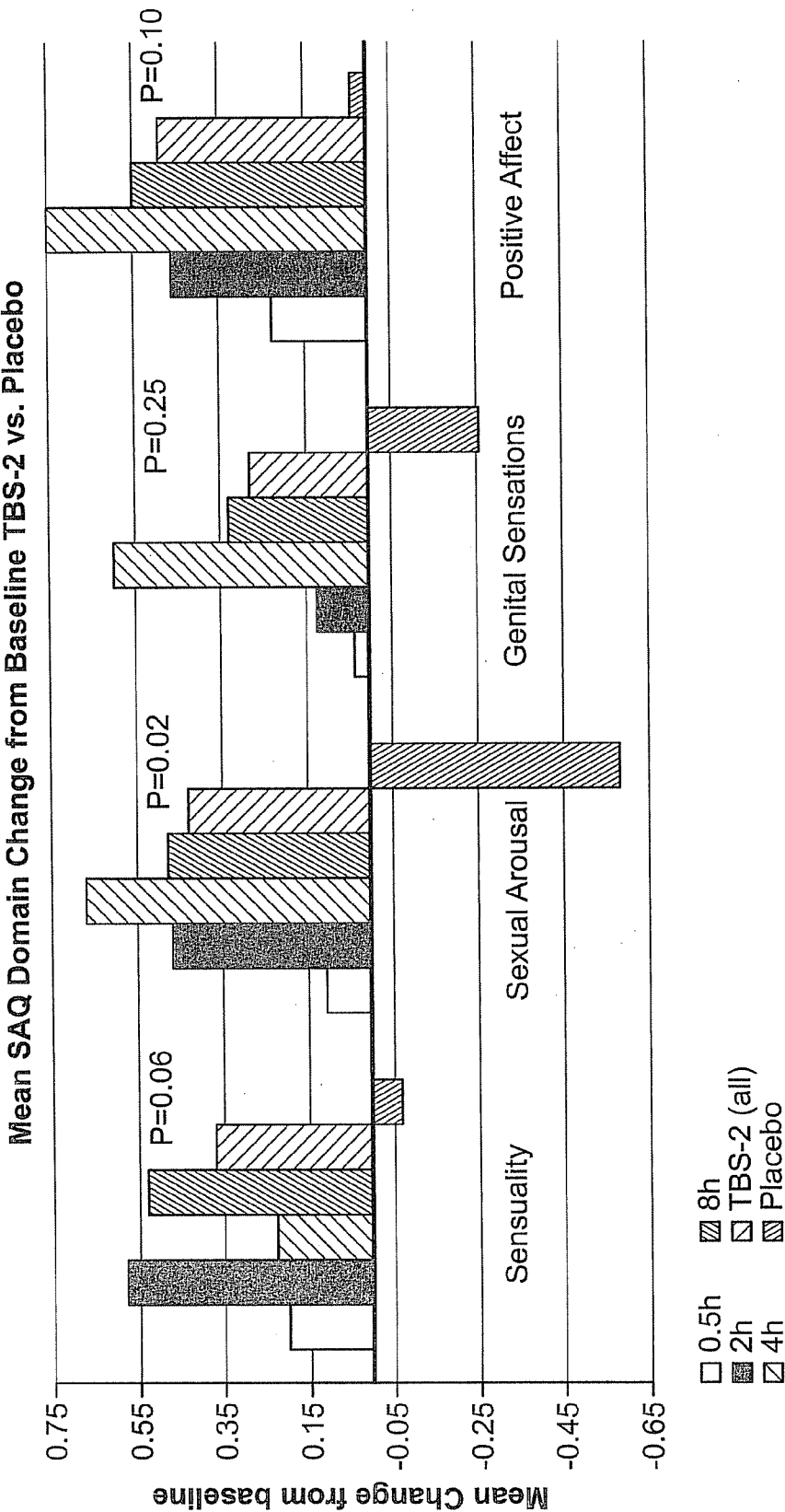


FIG. 17

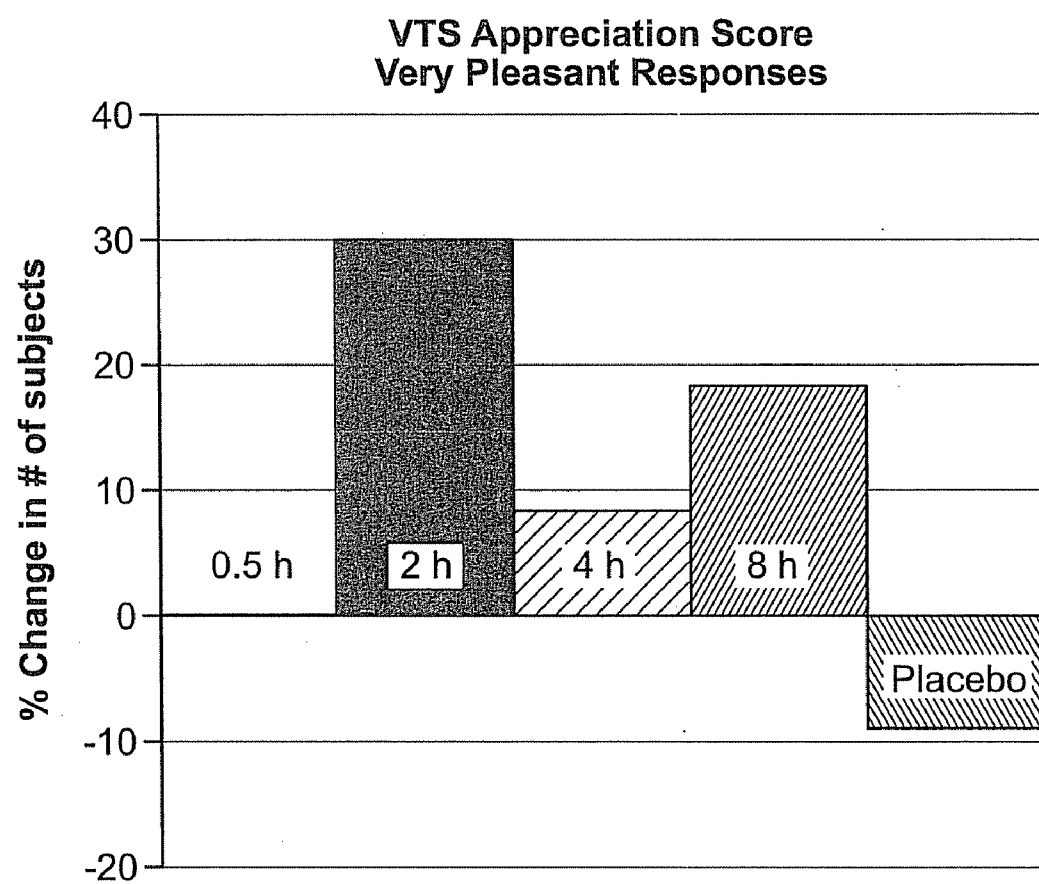


FIG. 18

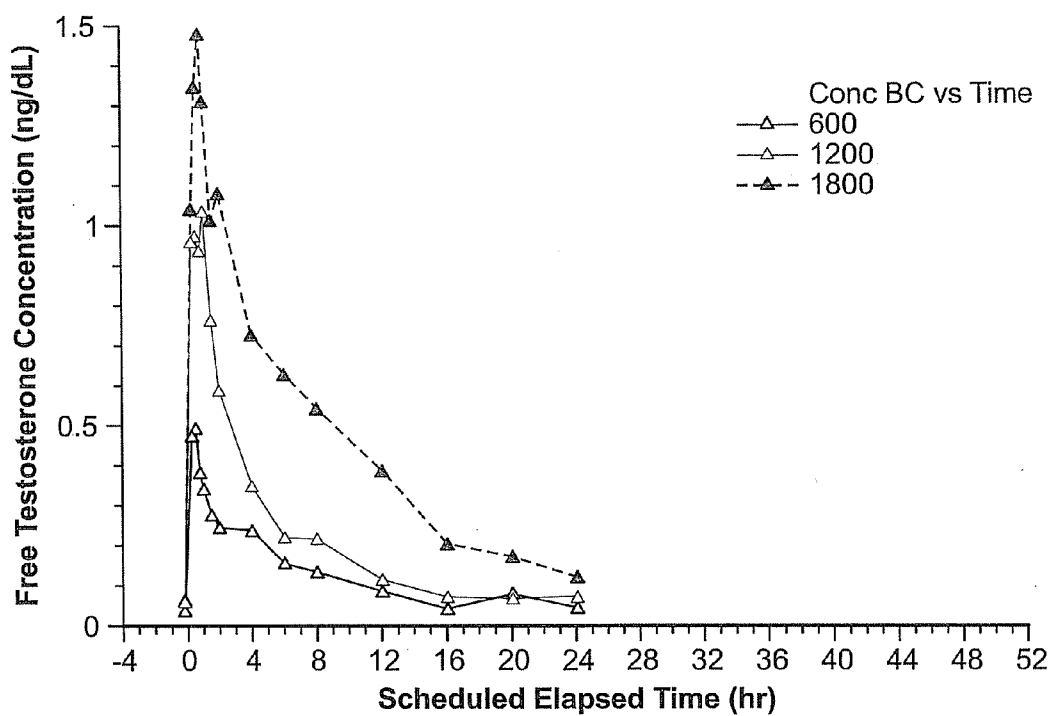


FIG. 19

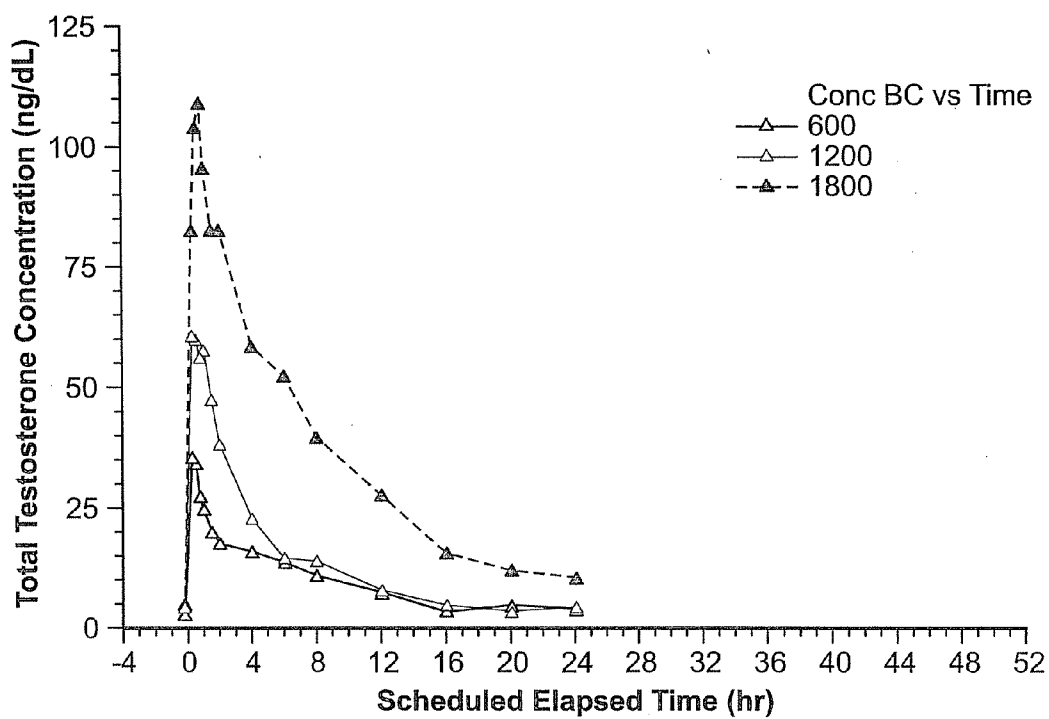


FIG. 20

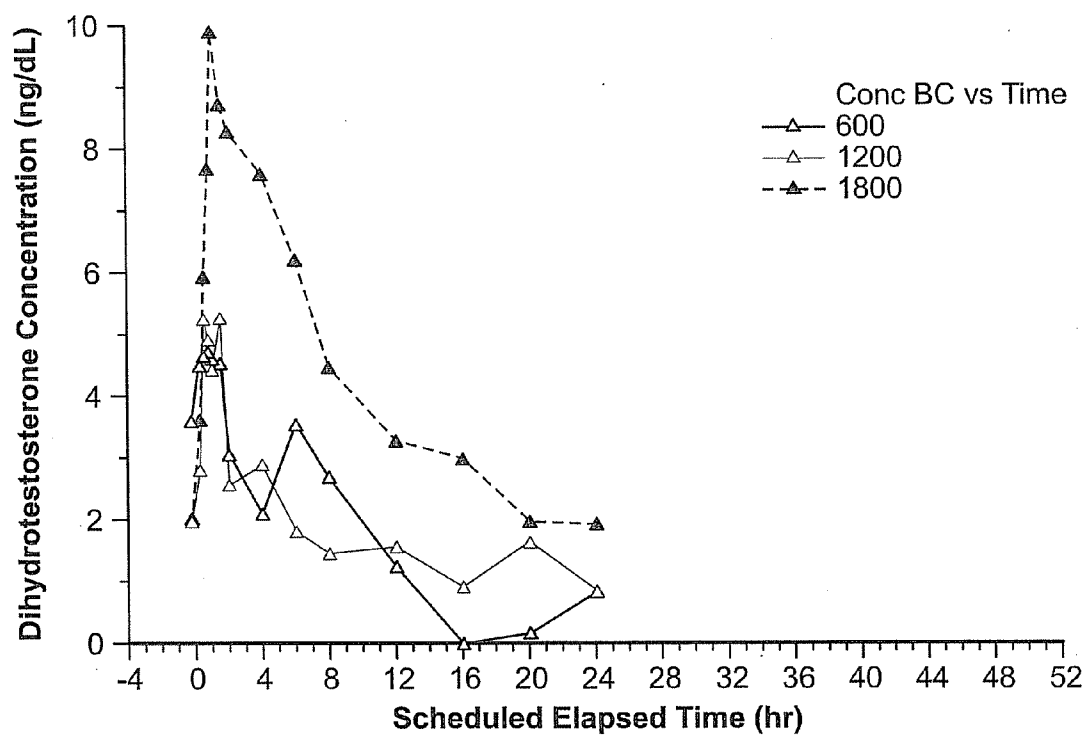


FIG. 21

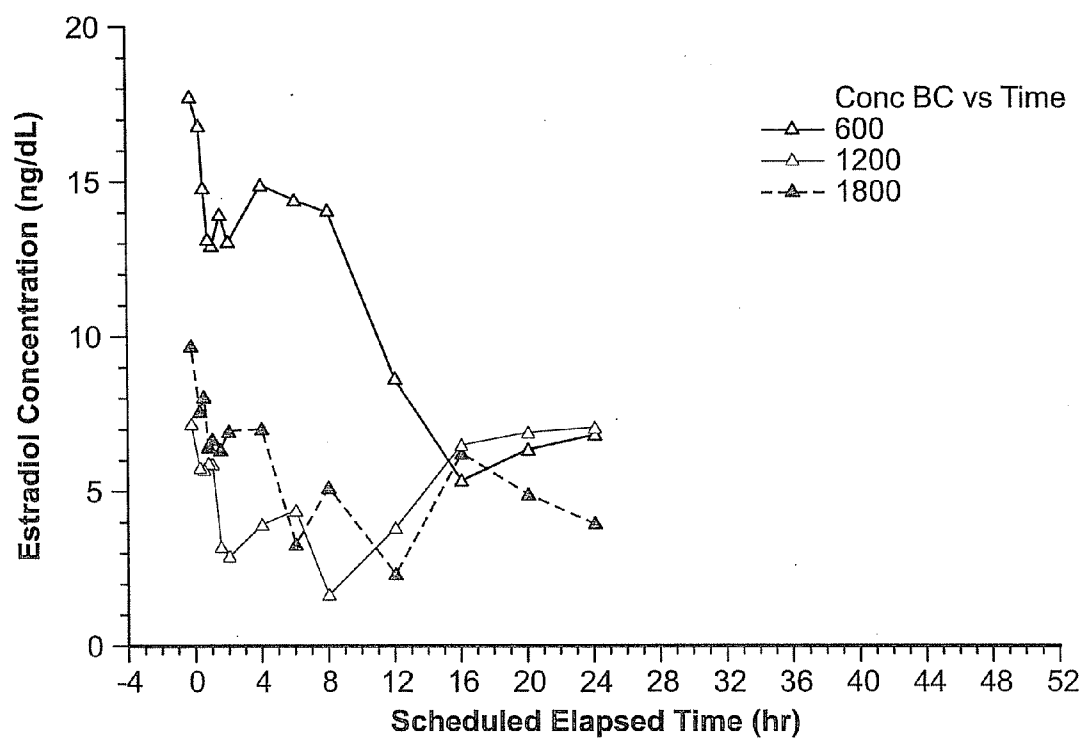


FIG. 22

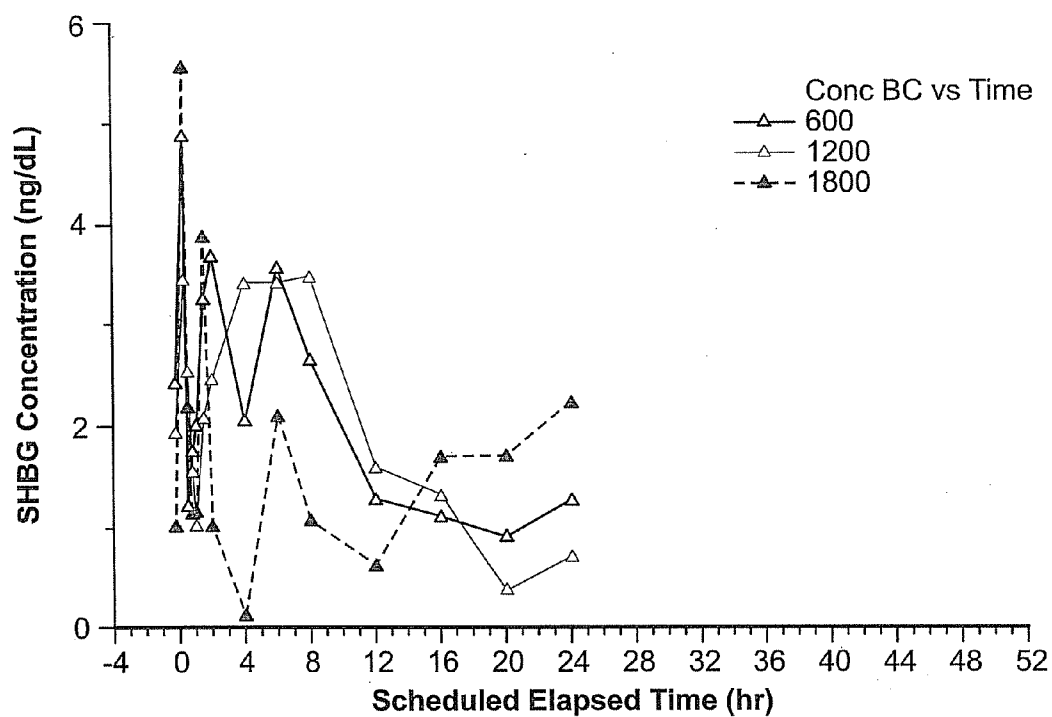


FIG. 23

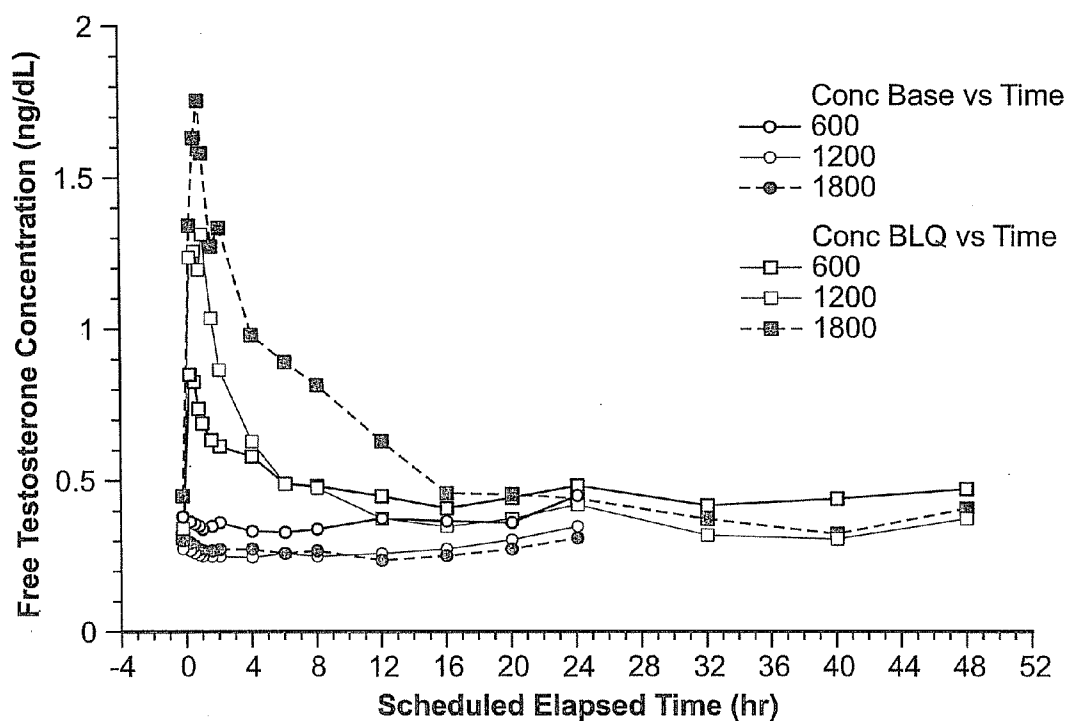


FIG. 24

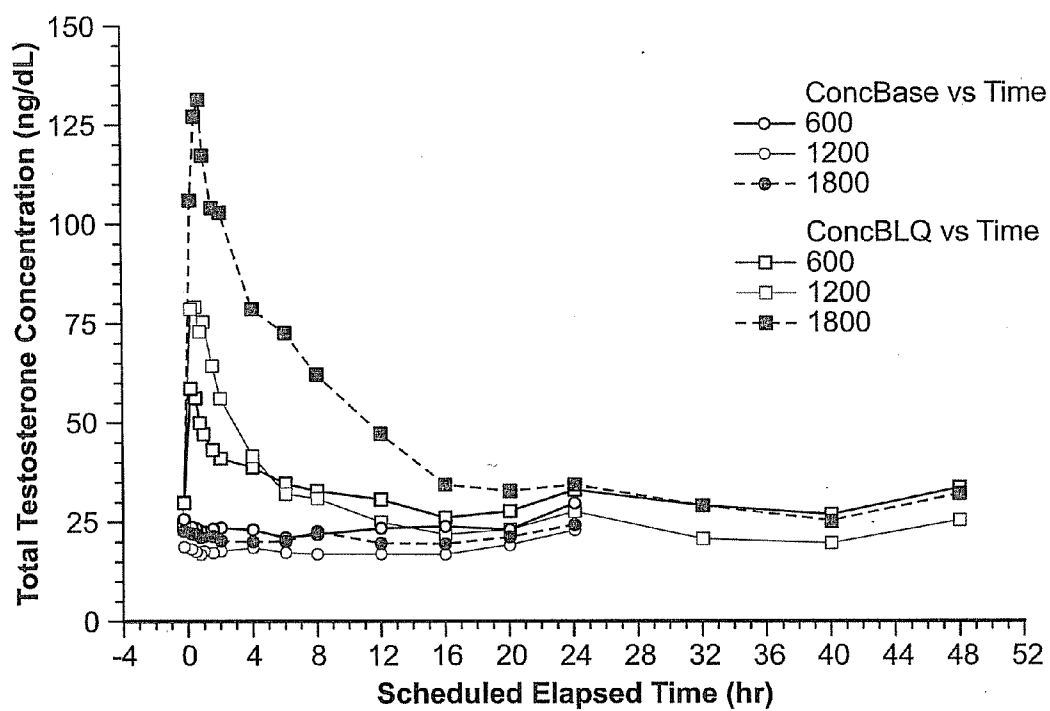


FIG. 25

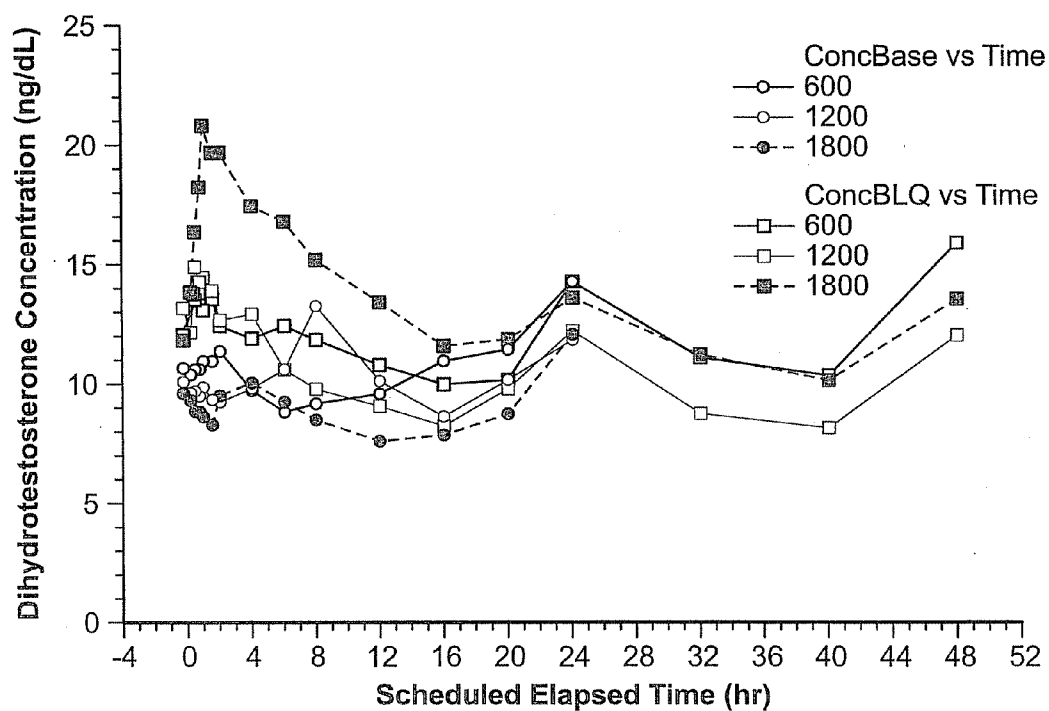


FIG. 26

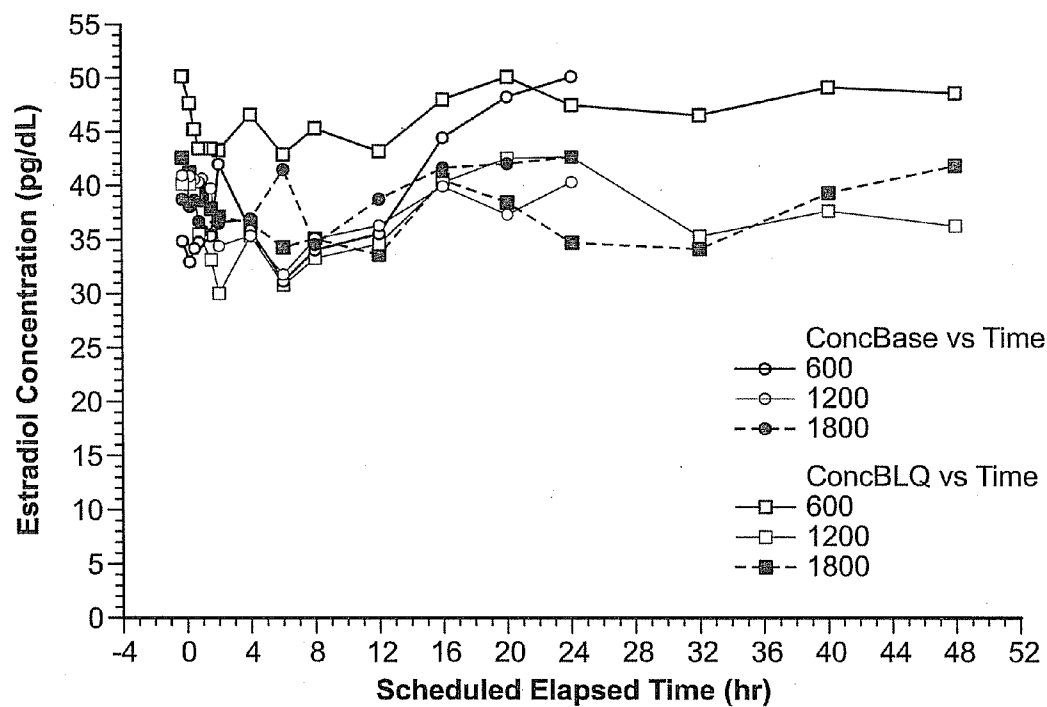


FIG. 27

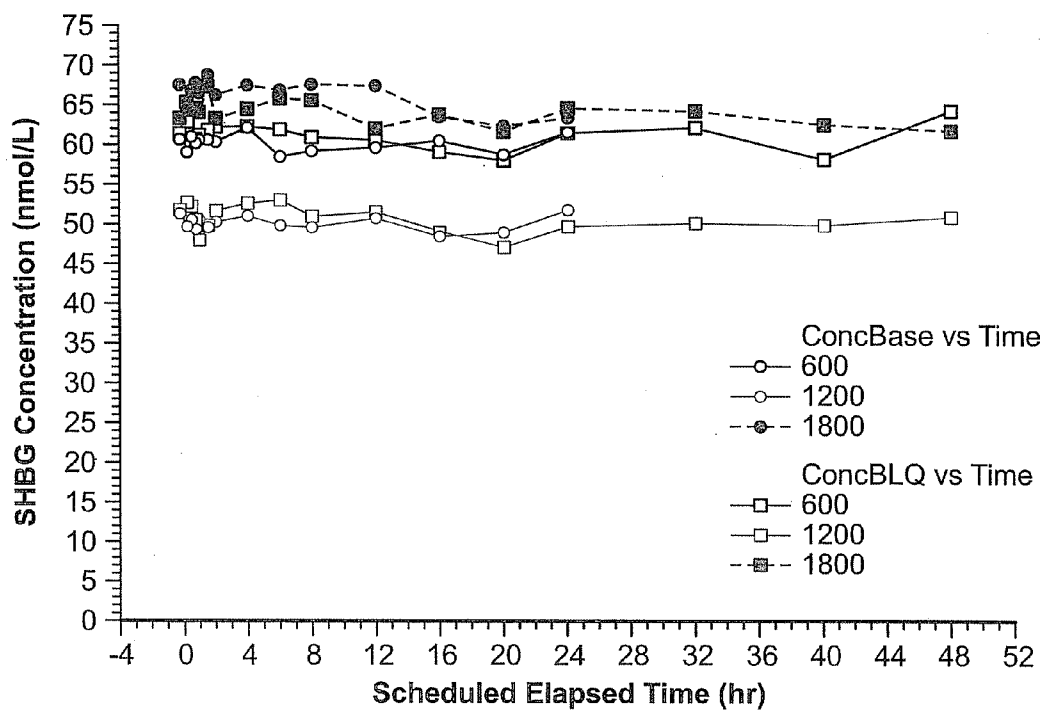


FIG. 28

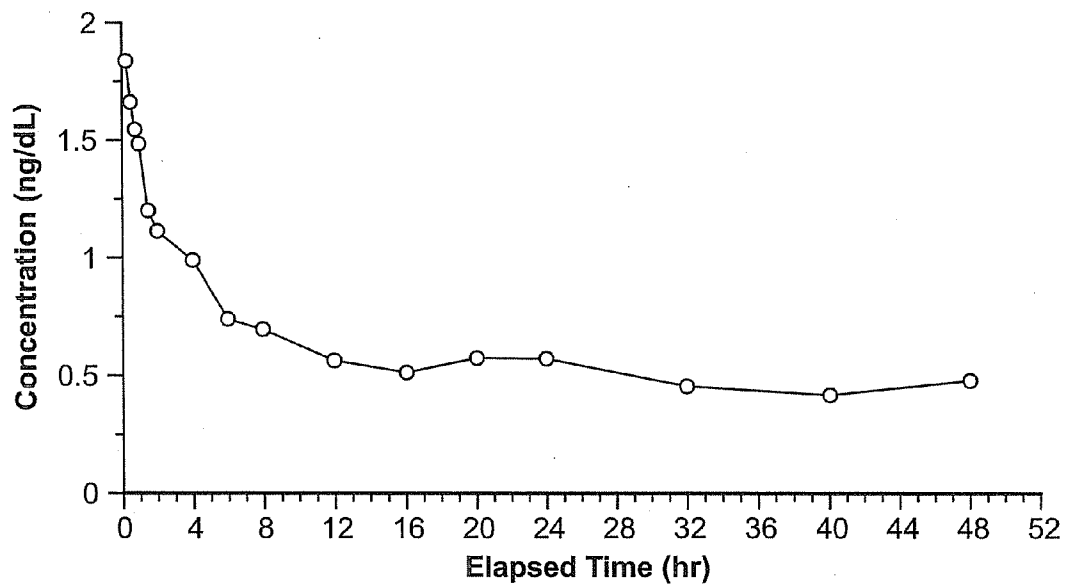


FIG. 29

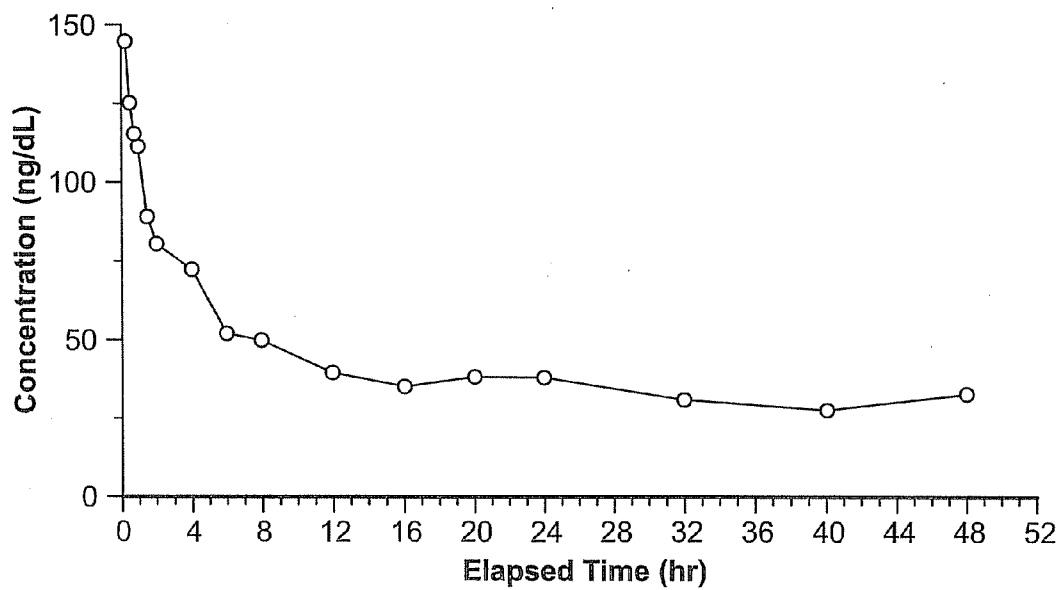


FIG. 30

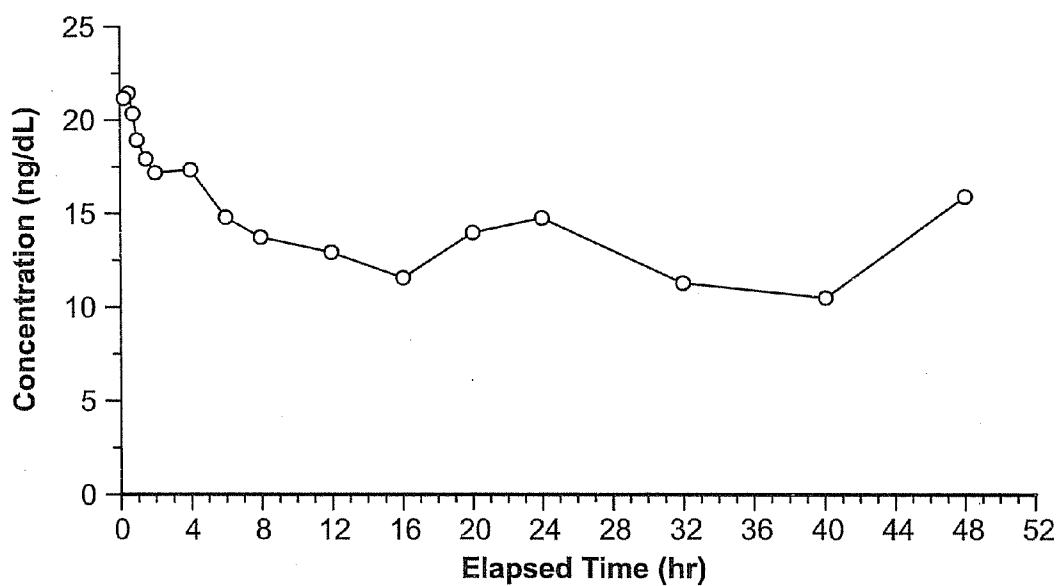


FIG. 31

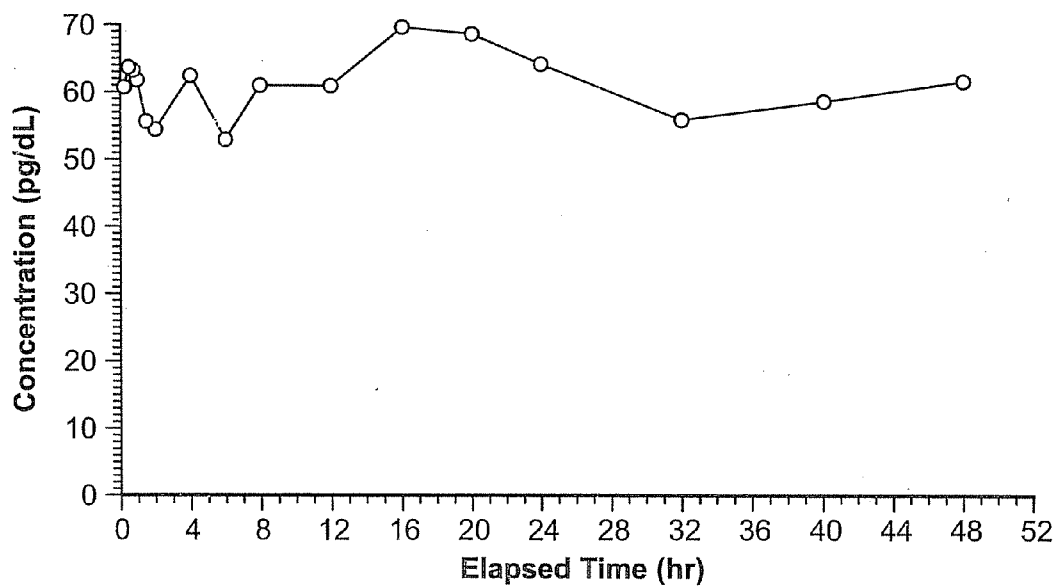


FIG. 32

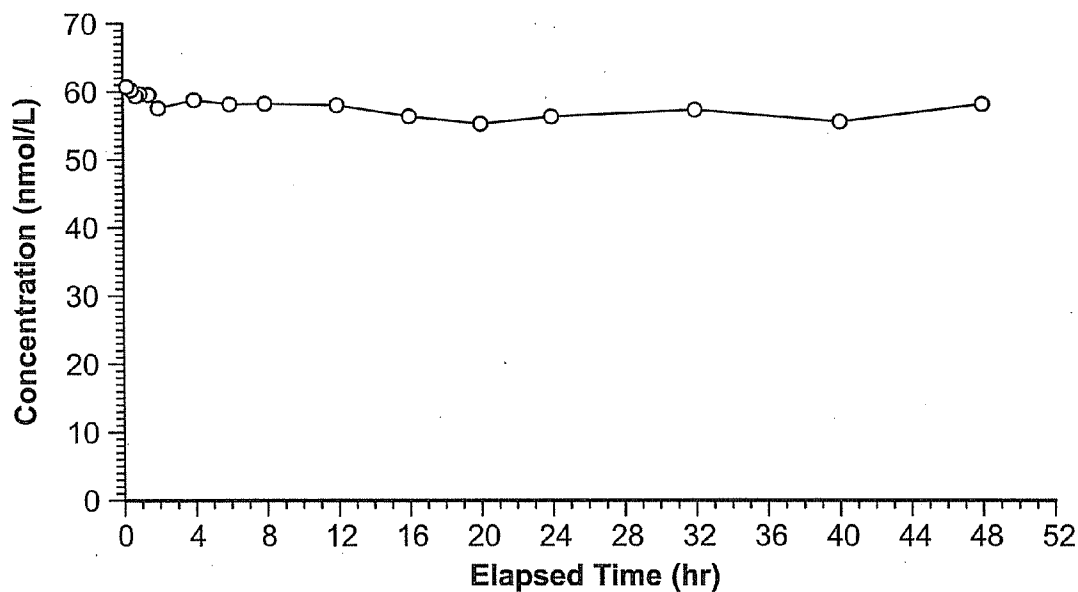


FIG. 33

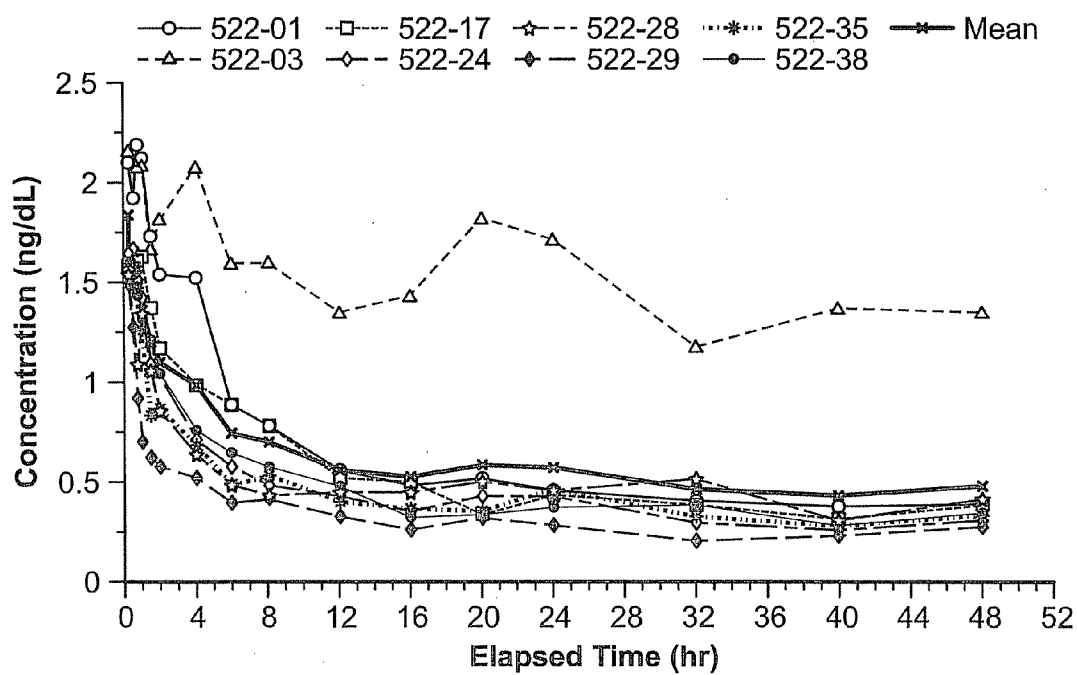


FIG. 34

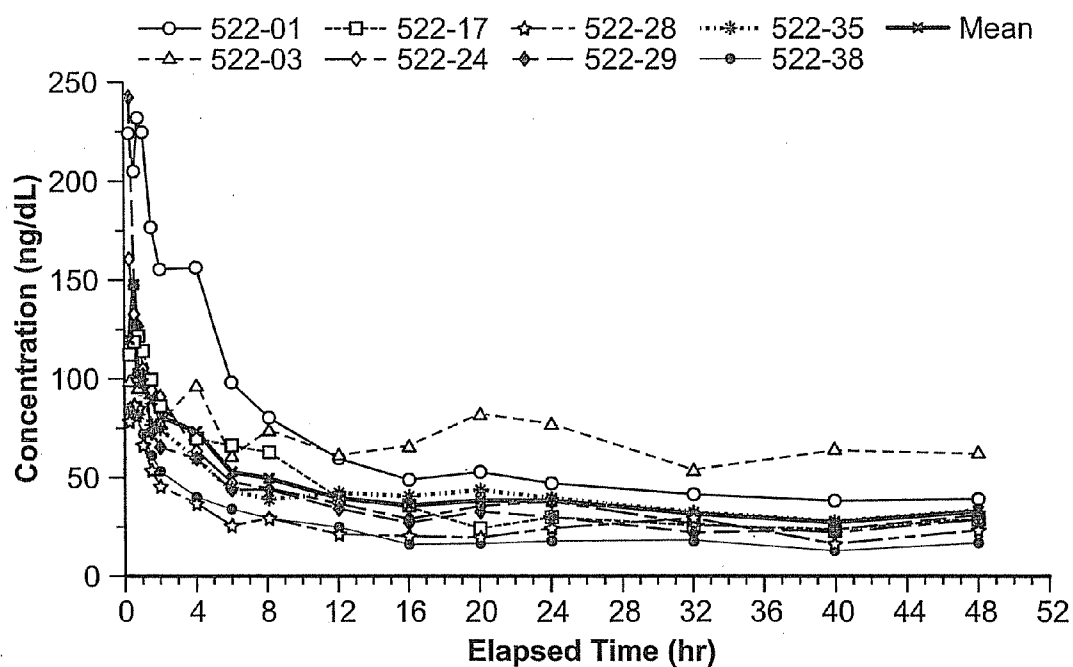


FIG. 35

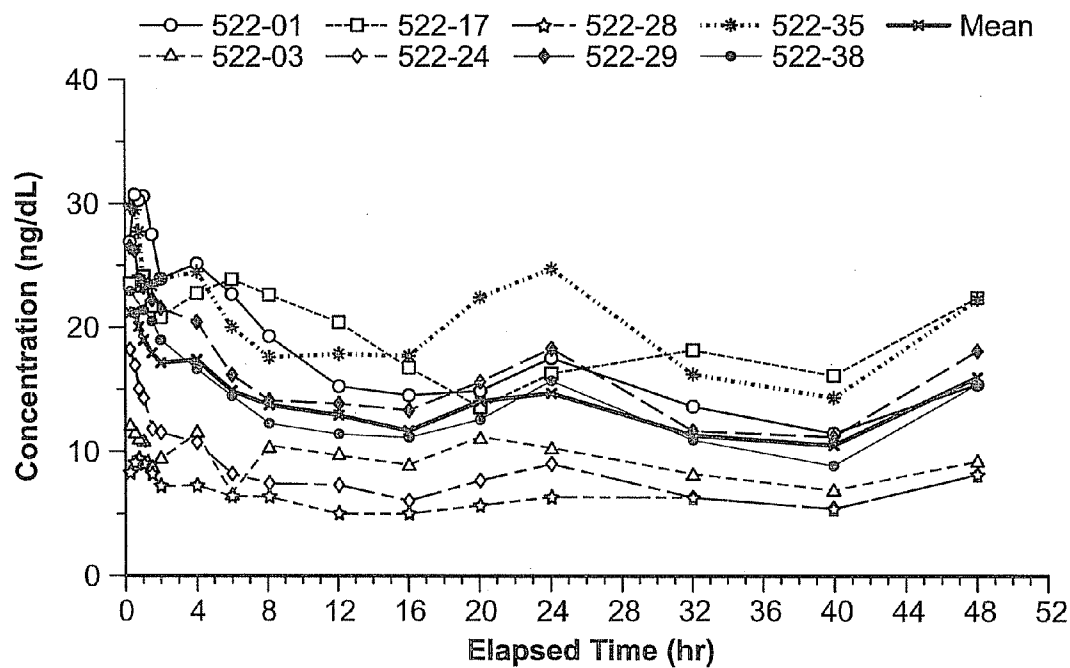


FIG. 36

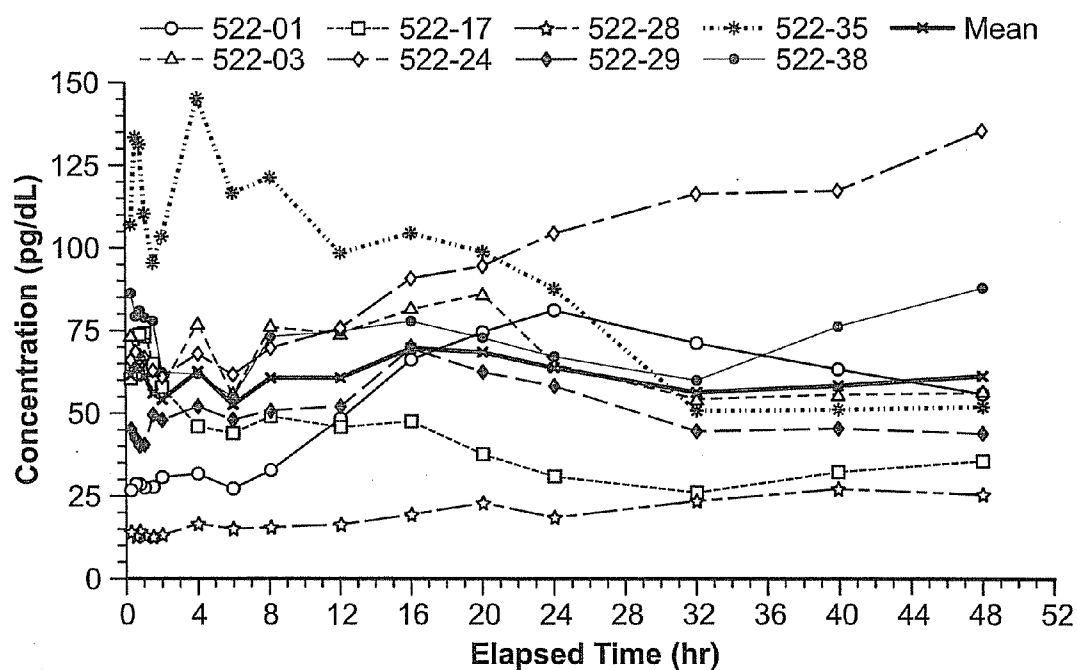


FIG. 37

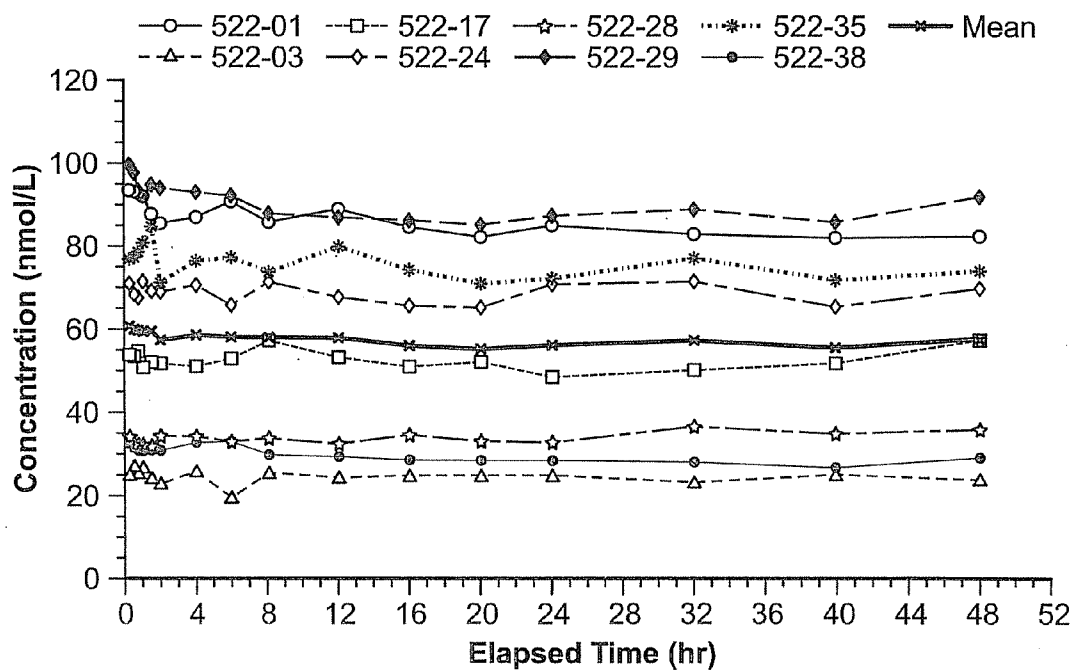


FIG. 38

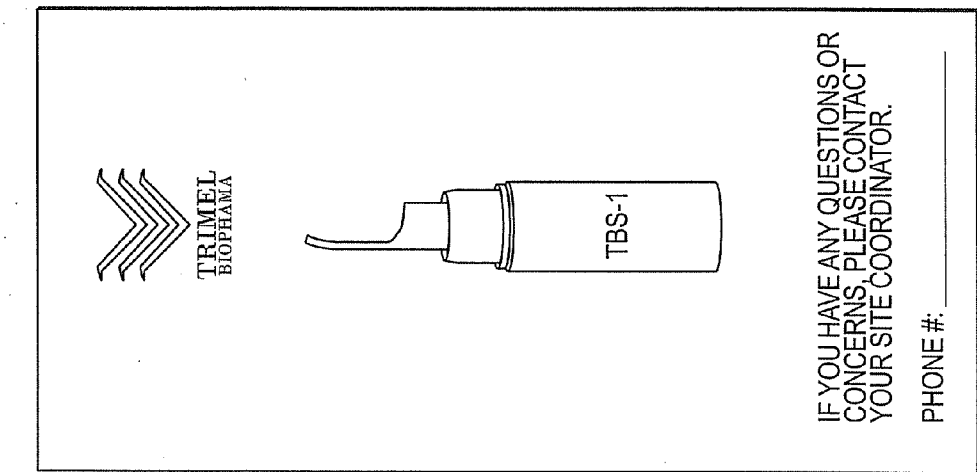
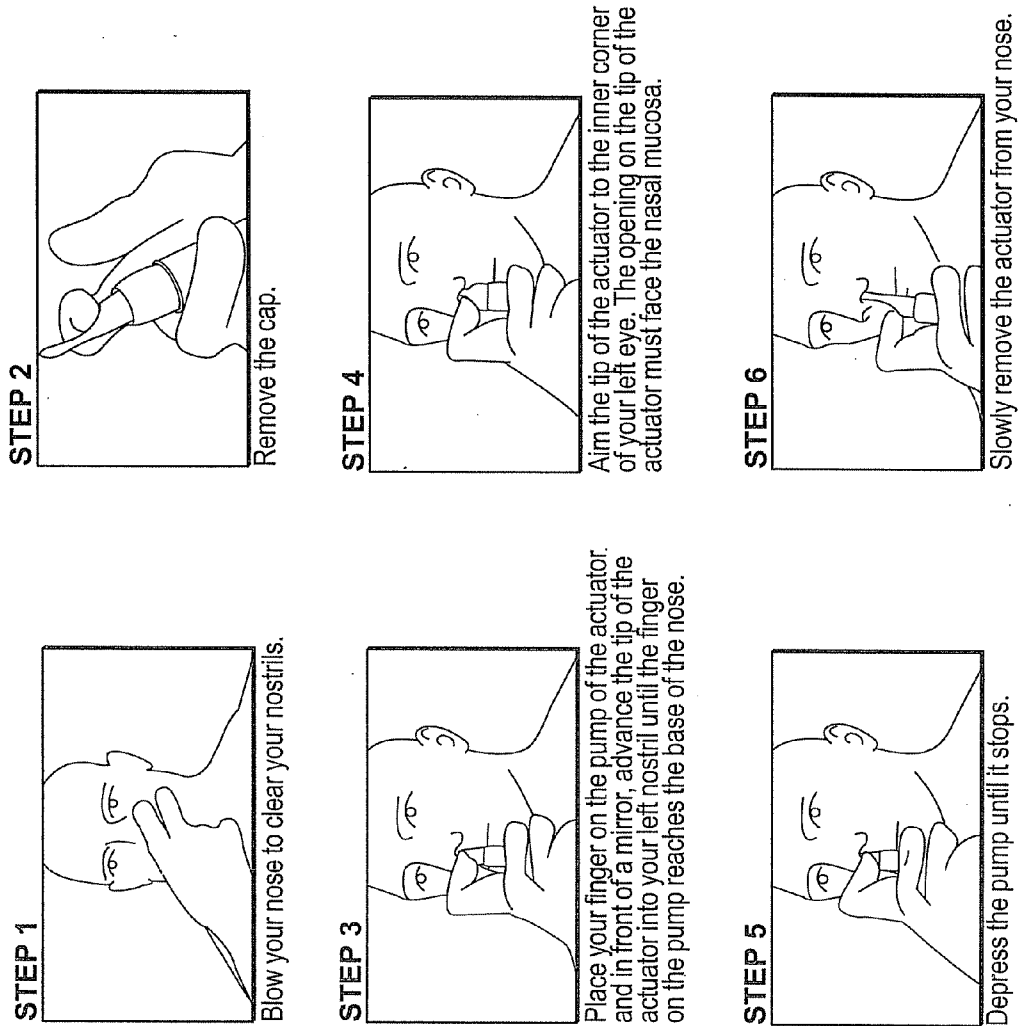


FIG. 39A



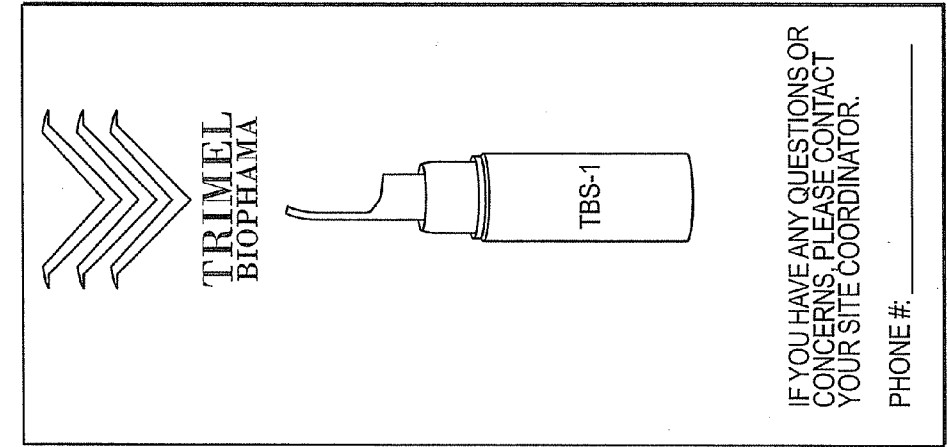
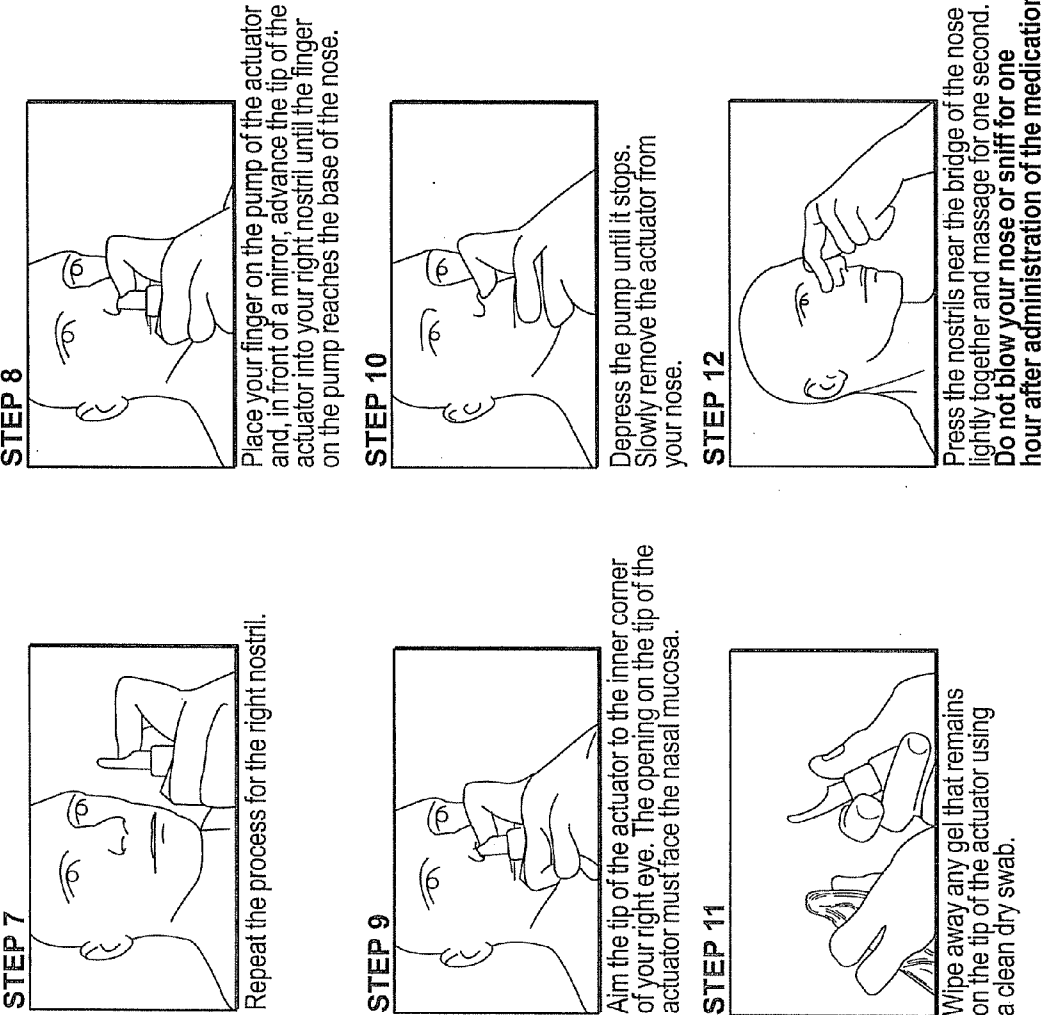


FIG. 39B



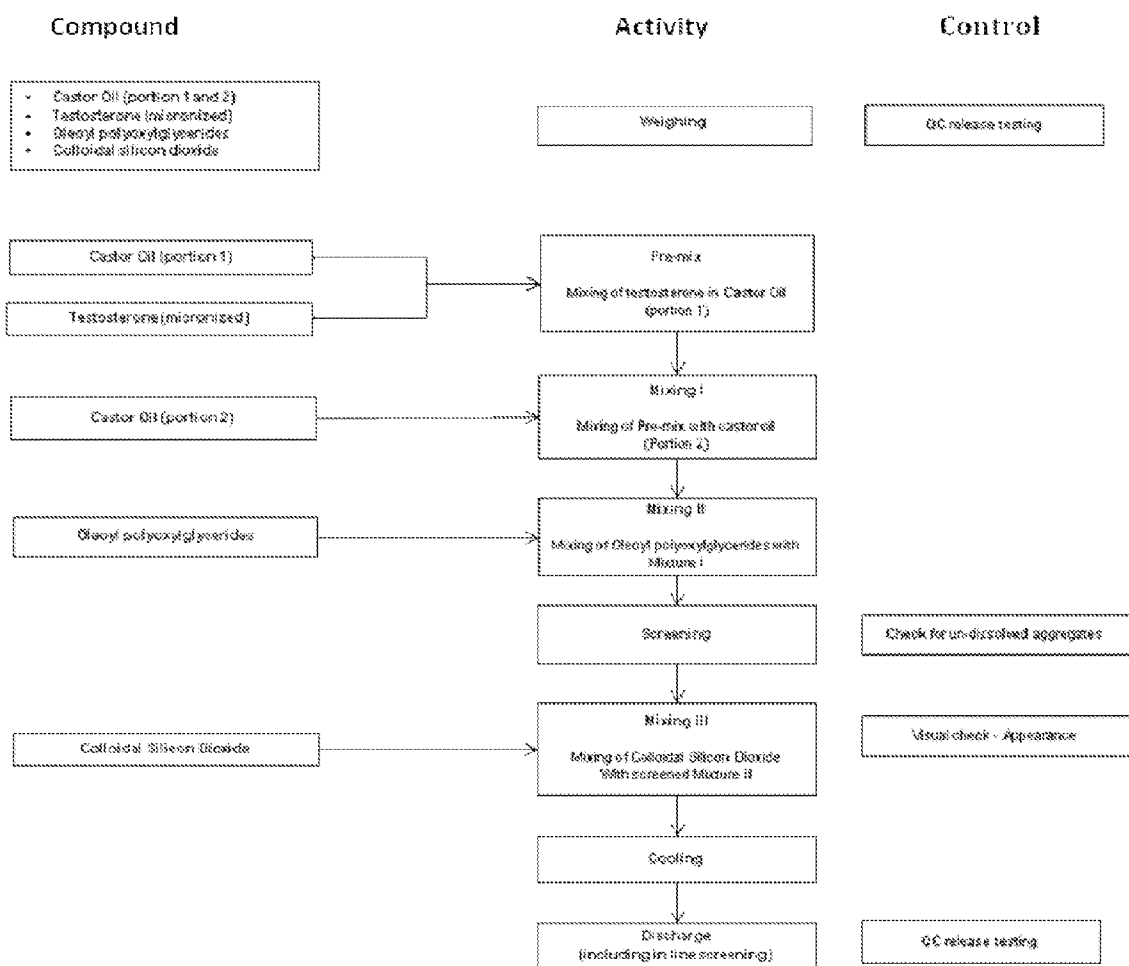


FIG. 40

**INTRANASAL 0.15% AND 0.24%
TESTOSTERONE GEL FORMULATIONS AND
USE THEREOF FOR TREATING
ANORGASMIA OR HYPOACTIVE SEXUAL
DESIRE DISORDER**

FIELD OF THE INVENTION

[0001] The present invention relates to lower dosage strength intranasal testosterone gels for providing intranasal delivery of testosterone to a female and intranasal treatment methods for treating females with anorgasmia and/or hypoactive sexual desire disorder (HSDD). In particular, the present invention relates to improved methods and lower dosage strength intranasal testosterone gel formulations for treating female anorgasmia and/or HSDD. The present invention also relates to a system for dispensing intranasally a precise dosage amount of such gels at an optimal anatomical location within each nostril of the female, so that an effective amount of testosterone is deposited within each nostril at the optimal anatomical location to effectively treat female anorgasmia and/or HSDD.

BACKGROUND

[0002] Reduced levels of endogenous steroid hormones in humans often lead to a variety of undesirable clinical symptoms. For example, low testosterone levels in men (hypogonadism) may result in clinical symptoms including impotence, lack of sex drive, muscle weakness, and osteoporosis. Similarly, in women, reduced levels of testosterone and/or estrogen may result in female sexual disorder, which include clinical symptoms such as lack of sex drive, lack of arousal or pleasure, decreased energy levels or fatigue with blunted motivation, flat mood or depression, reduced sense of well-being, insomnia, irritability, partial decreases in vaginal lubrication, and osteoporosis. Moreover, reduced levels of estrogen and/or progesterone in women, as observed during menopause, often result in clinical symptoms including hot flashes, night sweats, vaginal atrophy, decreased libido, and osteoporosis.

[0003] Testosterone has historically been thought of as a male hormone, but it is also synthesized in women in small amounts, primarily by the ovaries and adrenal glands. The physiological functions of testosterone in women include, among others, development of pubic and axillary hair, sexual libido; effects on bone density and muscle tone, sexual libido, and overall vitality and sense of psychological well-being. Testosterone plasma concentrations in pre-menopausal women normally fluctuate during the menstrual cycle, with the total testosterone plasma concentrations generally ranging between about 15 ng/dL and about 65 ng/dL. However, in the years leading to post-menopause, levels of circulating testosterone begin to decline, generally thought to be due to age-related reductions in ovarian and adrenal secretion. Generally, women with testosterone deficiency have total testosterone levels of less than about 20-25 ng/dL, while oophorectomized women can have testosterone levels of less than about 10 ng/dL.

[0004] In the National health and Social Life Survey of over 1,700 women aged 18-59, 43% acknowledged a form of female sexual dysfunction (FSD). See, e.g., Laumann et al.: Sexual dysfunction in the United States: prevalence and predictors; JAMA, 281: 537-544 (1999).

[0005] Hypoactive sexual desire disorder (HSDD), the most common women's sexual problem, is a condition characterized by the lack or absence of sexual fantasies and desire for sexual activity which causes marked distress or interpersonal difficulties. The sexual dysfunction is not accounted for by another psychiatric disorder nor is it a result of direct physiological effects of a substance (i.e., drug abuse) or a general medical condition.

[0006] Anorgasmia, the second most frequently reported women's sexual problem, is considered to be the persistent or recurrent delay in, or absence of, orgasm following a normal sexual excitement phase, causing marked distress or interpersonal difficulty. When a woman has sexual activity that is not accompanied by good quality orgasmic release, sexual activity may become a chore or a duty rather than a mutually satisfying, intimate experience. This may also lead to secondary loss of sexual interest and/or interpersonal difficulties.

[0007] Hypoactive sexual disorder disease and anorgasmia affect millions of women in the United States.

[0008] Sexual response is a complex and finely tuned process that can be disrupted at various time points in the reproductive life cycle (pre and postpartum, peri and postmenopausal) which likely accounts for the high prevalence of reported sexual dysfunction in the general population of healthy women. See, e.g., Laumann et al., Supra.

[0009] It is hypothesized that testosterone has central and peripheral effects on sexual function. The decline of androgen levels following surgically induced menopause has supported the hypothesis that a decrease in testosterone levels is related to a decrease in sexual desire. Testosterone, the primary circulation androgen in women, is a naturally occurring steroid. In women, androgens are derived from three sources: the adrenal glands, the ovaries and peripheral conversion. Androgens are secreted by the ovaries and the adrenal glands. Contrary to the sudden drop in estrogen during menopause, serum levels of androgens fall gradually as women age, primarily due to a decrease in the production of adrenal androgen precursors. See, e.g. Goldstat et al.: Transdermal testosterone therapy improves well-being, mood, and sexual function in pre-menopausal women; Menopause, 10(5): 390-398 (2003). As indicated above, this is likely due to a decline in ovarian and adrenal function with age.

[0010] A recent cross sectional study in woman ages 18-75 shows that total and free testosterone levels significantly decrease with age starting in the early reproductive years. In contrast to naturally occurring menopause, women who have undergone bilateral oophorectomy experience a dramatic decline in testosterone production with levels decreasing as much as 50%.

[0011] It has been reported that testosterone plays a role in mood, body composition, and bone mineral density and has central and peripheral effects on sexual function. See, e.g., Davis et al.: Androgen replacement in women: a commentary; Menopause, J Clin Endocrinol Metab, 84(6): 1886-1891 (1999); and Goldstat et al., Supra. In the periphery, testosterone is required for nitric oxide to stimulate vasocongestion for the engorgement of clitoral tissue and vaginal lubrication during sexual arousal.

[0012] Recent studies have shown that testosterone is effective in increasing the number of sexually satisfying events, increasing sexual desire and decreasing personal distress in bilaterally oophorectomized and hysterectomized women suffering from HSDD.

[0013] Central effects of testosterone are less well characterized. Testosterone stimulates dopamine release in various brain structures implicated in motivation and reward systems, including sexual desire. Testosterone was found to stimulate dopamine release in the medial preoptic area of the anterior hypothalamus under basal conditions and with sexual stimulation in rats. See, e.g., Halaris A.: Neurochemical aspects of the sexual response cycle; *CNS Spectrums*, 9: 211-216 (2003). An fMRI study in healthy women of different ages showed a testosterone level dependent modulation of amygdala activity, suggesting that an age-related decline in androgen levels contributes to the decrease in amygdala reactivity. In addition, it has been reported that a decreased amygdala reactivity in older women may be restored to levels of young women with intranasal exogenous testosterone. See e.g., van Wingen et al.: Testosterone increases amygdala reactivity in middle-aged women to a young adulthood level; *Neuropsychopharmacology*, February: 34(3) 539-547 (2009).

[0014] The use of androgens to increase women's sexual libido was reported in 1940 by Loeser. Salmon (1942) observed that a number of young, married women who formerly considered themselves "frigid" were able to experience "a marked increase in coital gratification, culminating in an orgasm" after testosterone propionate injections. The effects wore off within several weeks after the discontinuation of the injections. See, e.g., Traish et al.: Testosterone therapy in women with gynecological and sexual disorders: a triumph of clinical endocrinology from 1938 to 2008; *J Sex Med* 4:609-619 (2009). In the 1980s, the role of androgens in maintaining sexual function was studied in oophorectomized women. See, e.g., Sherwin et al.: The role of androgen in the maintenance of sexual functioning in oophorectomized women; *Psychosomatic Medicine*, 49:397-409 (1987). In the Sherwin et al. study, a three (3) month prospective open-label study of 44 women, it was reported that monthly injections of estrogen and testosterone increased rates of sexual desire, sexual arousal, and number of fantasies. It was further reported in the Sherwin et al. study that rates of intercourse and orgasm were higher in women treated with androgens and estrogen compared to the controls.

[0015] Over the past two decades, over 80 studies have been conducted in post-menopausal women with HSDD using exogenous testosterone through the oral, transdermal, sublingual or parental route of administration with or without concomitant estrogen therapy, in which some degree of an increase in sexual desire, arousal, frequency of satisfactory sexual activity, pleasure and responsiveness was allegedly observed. See, Traish et al. *Supra*, 2009.

[0016] Fewer studies have been performed in pre-menopausal women with low libido. Goldstat et al., *Supra*, apparently studied the effects of transdermal testosterone therapy on well-being, mood and sexual function in eugonadal, pre-menopausal women presenting with low libido. Testosterone therapy resulted in statistically significant improvements in the composite scores of the Psychological General Well-Being Index, the Sabbatsberg Sexual Self-Rating Scale and the Beck Depression Inventory when compared with placebo. These effects were found while the mean total testosterone levels were in the low end of the normal range before treatment, and at the high end of the normal range during treatment. On the different sub-scales of the Sabbatsberg Sexuality Scale, however, there was a significant effect of testosterone treatment on orgasm. This study suggests that,

although most previous studies with testosterone have addressed decreased sexual desire in post-menopausal women, there are also measurable effects in pre-menopausal women both on general sexual well-being and on orgasm specifically.

[0017] Intrinsa® is a testosterone slow-release transdermal patch. Intrinsa® is indicated for the treatment of hypoactive sexual desire disorder (HSDD) in bilaterally oophorectomized and hysterectomized (surgically induced menopause) women receiving concomitant estrogen therapy. Clinical studies using Intrinsa® have shown enhancement of sexual desire and number of satisfactory sexual events with mild androgenic skin effects as the primary safety concern in post-menopausal women with HSDD. See, e.g., Shifren et al.: Transdermal testosterone treatment in women with impaired sexual function after oophorectomy; *N Eng N Med*, 343(10): 682-688 (2000); Braunstein et al.: Safety and efficacy of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized placebo-controlled trial; *Arch Intern Med*, 165(14): 1582-1589 (2005); Buster et al.: Testosterone Patch for low sexual desire in surgically menopausal women: a randomized trial; *Obstet Gynecol*, 105(5 Pt 1): 944-952 (2005); Simon et al.: Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder; *J Clin Endocrinol Metab*, 90(9) 5226-5233 (2005); Davis et al.: Efficacy and safety of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial; *Menopause*, 13(3): 387-396 (2006); and Shifren et al.: Testosterone patch for the treatment of hypoactive sexual desire disorder in naturally menopausal women: results from the INTIMATE1 study; *Menopause*, 143:770-779 (2006).

[0018] LibiGel is a gel formulation of testosterone that is applied on the upper arm of a female. It is reported that treatment with LibiGel increases the number of satisfying sexual events versus baseline and placebo treated individuals. It is further reported that the effective dose of LibiGel produces testosterone blood levels within the normal range for pre-menopausal women. See, e.g., www.libigel.org.

[0019] Zestra® is a blend of botanical oils and extracts, including: Borage Seed Oil, Evening Primrose Oil, Angelica Extract, Coleus Forskohlii Extract, Theobromine, Anti-Oxidants {Ascorbyl Palmitate (Vitamin C), Tocopherol (Vitamin E)}; and Flavor (U.S. Pat. No. 6,737,084) that may benefit some women with anorgasmia. Zestra® has demonstrated significant improvements in the measures of desire, arousal and sexual satisfaction in women. See, e.g., www.zestra.com.

[0020] ArginMax™ is a mixture of L-arginine, ginseng, ginkgo, damiana, calcium, and iron. ArginMax™ for Women was formulated specifically for women. It contains calcium and iron to help relieve fatigue issues specific to women. The American ginseng in the men's product has been replaced with Damiana, an aromatic herb which helps calm anxiety and induce a relaxed state of mind. ArginMax™ for Women provides 100% of the RDA of vitamins A, C, E and the B-complex vitamins. ArginMax™ safely enhances the female sexual experience by improving circulation. Sufficient blood flow is critical to female arousal, engorgement and lubrication. See, e.g., www.arginimax.com.

[0021] In view of the fact that millions of women in the United States, as well as through out the world, suffer from HSDD and anorgasmia, there is a real and immediate need for an effective medical therapy that can treat these diseases, so

that the quality of life of these individuals can be improved. One therapeutic goal of such therapy to solve this immediate need might be to restore testosterone levels in women to young adulthood levels or to at least the natural pre-menopausal state in hopes to alleviate the symptoms generally associated with HSDD and/or anorgasmia due possibly to testosterone deficiency.

BRIEF SUMMARY OF THE INVENTION

[0022] The present invention overcomes the limitations and disadvantages associated with the treatment of anorgasmia and/or HSDD using available therapies through the discovery of novel lower dosage strength pernasal testosterone gels and methods of use to treat HSDD and/or anorgasmia. Particularly, the present invention overcomes the limitations and disadvantages of currently available options for administration of testosterone through the discovery of novel and improved lower dosage strength testosterone gel formulations specifically designed for intranasal administration to deliver therapeutically effective amounts of testosterone to treat females who suffer from and/or have been diagnosed with HSDD and/or anorgasmia.

[0023] The term “a therapeutically effective amount” means an amount of testosterone sufficient to induce a therapeutic or prophylactic effect for use in testosterone replacement or supplemental therapy to treat female sexual dysfunction (“FSD”), namely, hypoactive sexual desire disorder (“HSDD”) and/or female orgasmic disorder (“anorgasmia”) in females.

[0024] Thus, generally speaking, the present invention provides for new and improved, substantially less-irritating, lower dosage strength testosterone gel formulations formulated with testosterone in amounts ranging from between about 0.10% to about 1.5% by weight, for nasal administration to deliver a therapeutically effective amount of testosterone to effectively treat anorgasmia and/or HSDD.

[0025] The present invention is also directed to novel methods for pernasal administration of the nasal testosterone gels. Generally speaking, the novel methods involve depositing the intranasal testosterone gels topically into the nasal cavity of each nostril to deliver a therapeutically effective amount of testosterone, e.g., from about 150 mcg/nostril to about 600 mcg/nostril per application, over dose life for providing constant effective testosterone brain and/or blood levels for use in testosterone replacement or supplemental therapy, especially for effectively treating females in need of testosterone to treat anorgasmia and/or HSDD.

[0026] In accordance with the novel methods of the present invention, the intranasal testosterone gels are topically deposited on the outer external walls (opposite the nasal septum) inside the nasal cavity of each nostril, preferably at about the middle to about the upper section of the outer external wall (opposite the nasal septum) just under the cartilage section of the outer external wall inside the nasal cavity of each nostril. Once gel deposition is complete within each nostril of the nose, the outer nose is then gently and carefully squeezed and/or rubbed by the subject, so that the deposited gel remains in contact with the mucosal membranes within the nasal cavity for sustained release of the testosterone over dose life. Typical testosterone gel dosage amounts deposited pernasal application is between about 50 to about 150 microliters per nostril, and preferably about 100 microliters per nostril.

[0027] In carrying out the methods of the present invention, approximately between 50 microliters and about 150 micro-

liters of a lower dosage strength testosterone gel of the present invention is applied to each nostril of a subject once or twice daily, e.g., for one, two, three, four or more consecutive weeks, or for two, three, four, five or six consecutive days or more, or intermittently such as every other day or once, twice or three times weekly, or on demand once or twice during the same day, to treat HSDD and/or anorgasmia.

[0028] While the present invention has identified what it believes to be preferred concentrations of intranasal testosterone gel formulations, numbers of applications per day, durations of therapy, pernasal methods and pre-filled, multi-dose applicator systems, it should be understood by those versed in this art that any effective low dosage concentration of testosterone, i.e., between about 0.10% and about 1.5% by weight, in an intranasal gel formulation that delivers an effective amount of testosterone and any numbers of applications per day, week, month or year, as described herein, that can effectively treat anorgasmia and/or HSDD without causing unwanted testosterone treatment limiting reactions or related adverse events is contemplated by the present invention.

[0029] The present invention therefore provides for a new and improved treatment for anorgasmia and/or HSDD, wherein nasal administration of a lower dosage strength testosterone gel formulation of the present invention provides for: (1) rapid delivery of testosterone due to the highly permeable nasal tissue both systemically and across the blood-brain barrier into the brain; (2) fast onset of action; (3) avoidance of hepatic first-pass metabolism; (4) ease of administration to improve sexual experience; (5) avoidance of irritation from transdermal administration, particularly, no exposure to contacts, no transference from topical gels, and no local irritability from topical patch products; and (6) a more pleasant mode of administration, as compared to injections and buccal or sublingual tablets.

[0030] In other words, the present invention provides for a new and improved anorgasmia and/or HSDD treatment that (a) is easy and convenient to use either according to a prescribed treatment regimen or on-demand, (b) rapidly delivers therapeutically effective amounts of testosterone, thereby improving female sexual function in a timely manner, (c) provides for simple use, (d) has reduced side effects associated with prior exogenous systemic testosterone therapies, (e) avoids local irritability associated with prior topical gels and topical patches, and (f) eliminates the need for invasive and painful testosterone injections.

[0031] The present invention, in one embodiment, provides numerous surprising advantages over currently available therapies for anorgasmia and HSDD. For example, the present invention provides for (1) a rapid increase in the plasma testosterone plasma level (e.g., an increase in the plasma testosterone to a level of at least about 0.4 ng/ml within about 15 minutes immediately after nasal administration of the testosterone gel formulation of the invention); (2) a sustained increase in the plasma testosterone plasma level (e.g., an increase in the plasma testosterone level that is maintained in a subject for at least about 6 hours following nasal administration of the testosterone gel formulation of the invention); and (3) a higher maximum level of plasma testosterone as compared to the maximum level of plasma testosterone following administration of Intrinsa® within about 100 minutes immediately following administration (e.g., an increase in the plasma testosterone level to at least about 0.7 ng/ml as compared to about 0.1 ng/ml for Intrinsa®).

[0032] As demonstrated in FIG. 1, an improved testosterone gel formulation of the invention provide advantages over therapies for treating anorgasmia and HSDD that are currently available. For example, a testosterone gel formulation of the invention comprising about 0.6% testosterone by weight of the gel formulation is administered intranasally to subjects. In comparison, control subjects, who are treated with an Intrinsa® patch, receive a testosterone dose of about 2100-2800 mcg/day, up to approximately 3.5-4.5 times the testosterone received by women treated with the lower dosage strength testosterone gels of the present invention (i.e., about 600, 1800, or 2400 mcg/day, for the 0.15%, 0.45%, and 0.6% testosterone gels of the invention, respectively, or about 600, 1200 or 1800 mcg/day, for the 0.24%, 0.48% or 0.72% testosterone gels of the invention, respectively). Importantly, unlike the Intrinsa® patch, testosterone levels return to baseline after about 12 hours after treatment with the lower dosage strength testosterone gels of the present invention (at least for the 0.15% and 0.45% gel formulations of the invention).

[0033] One unique pharmacokinetic profile, as compared to Intrinsa®, is presented in FIG. 1. The improved 0.6% testosterone gel formulation for nasal administration of the present invention provides a plasma testosterone concentration, following nasal administration wherein (a) a plasma testosterone level of at least about 0.4 ng/ml is achieved; (b) a plasma testosterone level of at least about 0.7 ng/ml is achieved; (c) an increase in plasma testosterone level is achieved within at least about 10 minutes following nasal administration to a subject; (d) a plasma testosterone level of at least about 0.4 ng/ml is achieved and maintained for at least about 6 hours immediately following nasal administration to a subject; (e) a plasma testosterone level of at least about 0.3 ng/ml is achieved and maintained for at least about 13 hours immediately following nasal administration to a subject; and (f) a plasma testosterone level of at least about 0.7 ng/ml is achieved within about 100 minutes immediately following nasal administration to the subjects (see FIG. 1).

[0034] In contrast, following treatment with Intrinsa®, as illustrated in FIG. 1, the plasma testosterone level does not increase until at least about 3 hours following administration of Intrinsa®. Even then, a plasma testosterone concentration of at least about 0.4 ng/ml is not observed in the Intrinsa® treated subjects until at least about 6.5 hours following administration. The maximum plasma testosterone level of a subject treated with Intrinsa® (only about 0.68 ng/ml) is not observed until about 12 hours following administration of Intrinsa®.

[0035] Thus, one improved testosterone (0.6%) gel formulation for nasal administration of the present invention may provide one or more of the following plasma testosterone concentrations, following nasal administration:

[0036] (a) a plasma testosterone concentration of at least about 0.4 ng/ml within less than hour following administration;

[0037] (b) a plasma testosterone concentration of at least about 0.7 ng/ml within less than about 100 minutes following administration;

[0038] (c) a plasma testosterone concentration increase within at least about 10 minutes following nasal administration to a subject;

[0039] (d) a plasma testosterone concentration of at least about 0.4 ng/ml, wherein the increase is achieved and maintained for at least about 6 hours immediately following nasal administration to a subject;

[0040] (e) a plasma testosterone level of at least about 0.3 ng/ml is achieved and maintained for at least about 13 hours immediately following nasal administration to a subject; and/or

[0041] (f) a plasma testosterone concentration of at least about 0.7 ng/ml is achieved within about 100 minutes immediately following nasal administration to a subject (see FIG. 1).

[0042] Thus, as exemplified in FIG. 1, the present invention overcomes certain of the limitations associated with the treatment of anorgasmia and/or HSDD using currently available therapies, for example, Intrinsa®, and addresses current medical needs for (1) a pharmaceutical formulation that is conveniently, easily and unobtrusively administered; (2) a rapidly acting formulation that improves female sexual dysfunction in a timely manner; (3) a decrease in the incidence of application site reactions; (4) a formulation that has reduced side effects; and (5) a formulation that can be used either according to a prescribed treatment regimen or on demand; to treat anorgasmia and/or HSDD.

[0043] A safety study was also conducted in accordance with the present invention, as reported in Example 11, wherein women were treated intranasally with a 0.72% testosterone gel (about 1200 mcg/dose administration) of the present invention t.i.d. for two consecutive days and qd on the third consecutive day resulting in a total daily dose of testosterone of about 3600 mcg/day for the first two days and about 1200 mcg on the third consecutive day. As shown in Example 11, the intranasal testosterone gels of the present invention are believed to be safe and well tolerated.

[0044] The salient elements of the novel intranasal testosterone gels according to the present invention comprise (a) testosterone in a therapeutically effective amount, (b) a solvent, (c) a wetting agent, and (d) a viscosity increasing agent. The improved lower dosage strength testosterone gel formulations of the present invention may be formulated with testosterone in amounts by weight of between about 0.10% to about 1.5%, e.g., about 0.15%, 0.24%, 0.45%, 0.48%, 0.6% and 0.72%, and more preferably between about 0.24% and 0.72%. Exemplary nasally administered testosterone gel formulations contemplated by the present invention include:

[0045] (a) 0.15% testosterone, 91.85% castor oil, 4.0% oleoyl polyoxylglycerides, and 4% colloidal silicon dioxide;

[0046] (b) 0.24% testosterone, 91.76% castor oil, 4.0% oleoyl polyoxylglycerides, and 4% colloidal silicon dioxide

[0047] (c) 0.45% testosterone, 91.55% castor oil, 4.0% oleoyl polyoxylglycerides, and 4% colloidal silicon dioxide;

[0048] (d) 0.48% testosterone, 91.52% castor oil, 4.0% oleoyl polyoxylglycerides, and 4% colloidal silicon dioxide

[0049] (e) 0.6% testosterone, 91.4% castor oil, 4.0% oleoyl polyoxylglycerides, and 4% colloidal silicon dioxide; and

[0050] (f) 0.72% testosterone, 91.28% castor oil, 4.0% oleoyl polyoxylglycerides, and 4% colloidal silicon dioxide.

[0051] Thus, the improved testosterone gel formulations for nasal administration of the invention may further comprise any pharmaceutically acceptable vehicle, excipient and/or other active ingredient.

[0052] In addition, the present invention contemplates testosterone gel formulations for nasal administration that are pharmaceutically equivalent, therapeutically equivalent, bioequivalent and/or interchangeable, regardless of the method selected to demonstrate equivalents or bioequiva-

lence, such as pharmacokinetic methodologies, microdialysis, in vitro and in vivo methods and/or clinical endpoints described herein.

[0053] Thus, the present invention contemplates testosterone gel formulations for nasal administration that are bioequivalent, pharmaceutically equivalent and/or therapeutically equivalent, especially testosterone gel formulations for nasal administration that are 0.15% testosterone by weight of the gel formulation, 0.24% testosterone by weight of the gel formulation, 0.45% testosterone by weight of the gel formulation, 0.48% testosterone by weight of the gel formulation, 0.6% testosterone by weight of the gel formulation and 0.72% testosterone by weight of the gel formulation, when used in accordance with the therapy of the present invention to treat anorgasmia and/or HSDD by intranasal administration. Thus, the present invention contemplates: (a) pharmaceutically equivalent testosterone gel formulations for nasal administration which contain the same amount of testosterone in the same dosage form; (b) bioequivalent testosterone gel formulations for nasal administration which are chemically equivalent and which, when administered to the same individuals in the same dosage regimens, result in comparable bioavailabilities; (c) therapeutic equivalent testosterone gel formulations for nasal administration which, when administered to the same individuals in the same dosage regimens, provide essentially the same efficacy and/or toxicity; and (d) interchangeable testosterone gel formulations for nasal administration of the present invention which are pharmaceutically equivalent, bioequivalent and therapeutically equivalent.

[0054] While the intranasal testosterone gels of the present invention are preferred pharmaceutical preparations when practicing the novel methods of the present invention, it should be understood that the novel topical intranasal gel formulations and methods of the present invention also contemplate the pernasal administration of any suitable active ingredient, either alone or in combination with testosterone or other active ingredients, such as neurosteroids or sexual hormones (e.g., androgens and progestins, like testosterone, estradiol, estrogen, oestrone, progesterone, etc.), neurotransmitters, (e.g., acetylcholine, epinephrine, norepinephrine, dopamine, serotonin, melatonin, histamine, glutamate, gamma aminobutyric acid, aspartate, glycine, adenosine, ATP, GTP, oxytocin, vasopressin, endorphin, nitric oxide, pregnenolone, etc.), prostaglandin, benzodiazepines like diazepam, midazolam, lorazepam, etc., and PDE5 inhibitors like sildenafil, tadalafil, vardenafil, etc., in any suitable pharmaceutical preparation, such as a liquid, cream, ointment, salve or gel. Examples of additional topical formulations for practice in accordance with the novel methods of the present invention include the topical pernasal formulations disclosed in, for example, U.S. Pat. Nos. 5,578,588, 5,756,071 and 5,756,071 and U.S. Patent Publication Nos. 2005/0100564, 2007/0149454 and 2009/0227550, all of which are incorporated herein by reference in their entireties.

[0055] The present invention is also directed to packaged pharmaceuticals comprising the novel and improved testosterone gel formulations for nasal administration of the invention. For example, the present invention contemplates pre-filled, single or multi-dose applicator systems for pernasal administration to strategically and uniquely deposit the nasal testosterone gels at the preferred locations within the nasal cavity for practicing the novel methods and teachings of the present invention. Generally, speaking the applicator sys-

tems of the present invention are, e.g., airless fluid, dip-tube fluid dispensing systems, pumps, syringes or any other system suitable for practicing the methods of the present invention. The applicator systems or pumps include, for example, a chamber, pre-filled with a single dose or multiple doses of an intranasal testosterone gel of the present invention, that is closed by an actuator nozzle or cap. The actuator nozzle may comprise an outlet channel and tip, wherein the actuator nozzle is shaped to conform to the interior surface of a user's nostril for (a) consistent delivery of uniform dose amounts of an intranasal testosterone gel of the present invention during pernasal application within the nasal cavity, and (b) deposition at the instructed location within each nostril of a patient as contemplated by the novel methods and teachings of the present invention. Examples of pre-filled, multi-dose applicator systems include, e.g., (a) the COMOD system available from Ursatec, Verpackung-GmbH, Schillerstr. 4, 66606 St. Wendel, Germany, (b) the Albion or Digital airless applicator systems available from Airlessystems, RD 149 27380 Charleval, France or 250 North Route 303 Congers, N.Y. 10950 (See, for example, FIG. 39), (c) the nasal applicators from Neopac, The Tube, Hoffmann Neopac AG, Burgdorfstrasse 22, Postfach, 3672 Oberdiessbach, Switzerland, or (d) the syringes described in the Examples herein below.

[0056] It should be understood by those versed in this art that the amount of testosterone in a lower dosage strength intranasal testosterone gel of the present invention that will be therapeutically effective in a specific situation will depend upon such things as the dosing regimen, the application site, the particular gel formulation, dose longevity and the condition being treated. As such, it is generally not practical to identify specific administration amounts herein; however, it is believed that those skilled in the art will be able to determine appropriate therapeutically effective amounts based on the guidance provided herein, information available in the art pertaining to testosterone replacement therapy, and routine testing.

[0057] It should be further understood that the above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description further exemplifies illustrative embodiments. In several places throughout the specification, guidance is provided through examples, which examples can be used in various combinations. In each instance, the examples serve only as representative groups and should not be interpreted as exclusive examples.

BRIEF DESCRIPTION OF THE DRAWINGS

[0058] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0059] The foregoing and other objects, advantages and features of the present invention, and the manner in which the same are accomplished, will become more readily apparent upon consideration of the following detailed description of the invention taken in conjunction with the accompanying figures and examples, which illustrate embodiments, wherein:

[0060] FIG. 1 shows the effects of a single dose testosterone nasal gel formulation of the invention (0.6% testosterone by weight of the gel formulation) as compared to Intrinsa® on the plasma testosterone levels in subjects diagnosed with

HSDD (triangles—100 microliters 0.6% testosterone nasal gel formulation of the invention in each nostril; squares—Intrinsa®);

[0061] FIG. 2 shows the effects of a testosterone nasal gel formulation of the invention after the fifth 12 hourly dose (0.6% testosterone by weight of the gel formulation) as compared to Intrinsa® on the plasma testosterone levels in subjects diagnosed with anorgasmia (TBS-2 line-0.6% testosterone nasal gel formulation of the invention (100 mcI*2); solid line-Intrinsa®);

[0062] FIG. 3 shows the effects of a testosterone nasal gel formulation of the invention (0.15, 0.45 and 0.6% testosterone by weight of the gel formulation) after the first and the 5th 12 hourly dose as compared to Intrinsa® single dose on the plasma testosterone levels in subjects diagnosed with HSDD;

[0063] FIG. 4 shows the effects of a testosterone nasal gel formulation of the invention at day zero (open triangles) and day 3 (dose 5—closed square—of 0.6% testosterone by weight of the gel formulation) as compared to Intrinsa® on the plasma testosterone levels in subjects diagnosed with HSDD (open squares day 0—lines day 3 of 0.6% squares—Intrinsa®);

[0064] FIG. 5 is a copy of FIG. 1, but with comparative data for the 0.6% testosterone gel and Intrinsa® from days 1 and 3;

[0065] FIG. 6 is comparative data from treatment study for HSDD for the 0.6% testosterone gel and Intrinsa® from days 1 and 3;

[0066] FIG. 7 shows the effects of a testosterone nasal gel formulation of the invention (0.15, 0.45 and 0.6% testosterone by weight of the gel formulation) after the first and the 5th 12 hourly dose as compared to Intrinsa® single dose on the plasma testosterone levels in subjects diagnosed with anorgasmia;

[0067] FIG. 8 shows the effects of a testosterone nasal gel formulation of the invention (0.45% day 3 (Med Day 1) and 0.6% day zero and Day 3 (ANOR) testosterone by weight of the gel formulation) after the first and the 5th 12 hourly dose;

[0068] FIG. 9 shows the accumulation effect effects of a testosterone nasal gel formulation of the invention (0.15, 0.45 and 0.6% testosterone by weight of the gel formulation) before each bi-daily dose of testosterone gel on the plasma testosterone levels at time (0) in subjects diagnosed with HSDD or anorgasmia;

[0069] FIG. 10 (same as 12 but table on AUC Cavg and Cmax) vs Intrinsa® (note—because Intrinsa® is not approved for Anorgasmia, it was not assessed in the study);

[0070] FIG. 11 presents trough data for subjects diagnosed with anorgasmia or HSDD and treated with testosterone nasal gel formulations of the invention (0.15%, 0.45% or 0.6% testosterone by weight of the gel formulation) or placebo (anorgasmia) or Intrinsa® (HSDD);

[0071] FIG. 12 presents distribution of testosterone mean concentration for TBS-2 treatments for the first (AUC₀₋₁₂) and the last dose (AUC₄₈₋₆₀). The boxplots show the median (thick solid line), the inter-quartile range (box) and the extreme values (whiskers). The horizontal solid grey line indicates the median C_{mean} during treatment with Intrinsa from 48 to 60 hrs, and the horizontal dotted lines indicate the minimum and maximum C_{mean} during treatment with Intrinsa from 48-60 hrs;

[0072] FIG. 13 presents scores on the AFSDQ 30 minutes (left) and 4.5 hours (right) after dosing. White bars=start of session; solid bars=end of session. Groups left to right:

ANOR placebo, ANOR low, ANOR medium, ANOR high, HSDD Intrinsa patch (at 30 min. only), HSDD low, HSDD medium, HSDD high;

[0073] FIG. 14 concerns mean testosterone levels following TBS-2 high, TBS-2 medium and TBS-2 low dose administration or placebo administration (hours 0-12) in women with anorgasmia. In this pharmacokinetic study, as described in Example 9, three different dosage strengths of TBS-2 testosterone bio-adhesive gel formulations of the invention are administered intranasally to a hybrid group of 12 healthy and anorgasmic women. As illustrated in FIG. 14, the testosterone serum levels are compared for the three different testosterone bio-adhesive gel formulations of the invention (0.15%, 0.45% and 0.6% testosterone by weight of the gel formulation, as reported in Examples 1-5), during 2 hours following a single application of each TBS-2 formulation or a placebo to each of the 12 women. The total testosterone dosage strength that is administered is either 1.2 mg (0.6%-0.6 mg/100 µl/nos-tril), 0.9 mg (0.45%-0.45 mg/100 µl/nos-tril) or 0.3 mg (0.15%-0.15 mg/100 µl/nos-tril). Following administration, the testosterone serum level is measured and compared. As shown in FIG. 14, the C_{max} and C_{avg} for testosterone following single dose administration for each of the three dosage strengths do not exceed the normal testosterone serum level in women (3-80 ng/dL);

[0074] FIG. 15 depicts a study design (Example 10), in which 56 anorgasmic women are enrolled for a Vibrotactile Stimulation Study (VTS) that concerns the three different testosterone bio-adhesive gel formulations of the invention, i.e., the 0.15%, 0.45% and 0.6% testosterone by weight of the gel formulation, as reported in Examples 1-5;

[0075] FIG. 16 depicts orgasm result of the VTS study of Example 10, wherein the number of orgasms achieved during the treatment phase and the post-treatment phase are compared;

[0076] FIG. 17 depicts sexual response results of the VTS study of Example 10 for the three different testosterone bio-adhesive gel formulations of the invention, i.e., the 0.15%, 0.45% and 0.6% testosterone by weight of the gel formulation, as reported in Examples 1-5, as compared to placebo;

[0077] FIG. 18 depicts the VTS appreciation score of the VTS study of Example 10 for the three different testosterone bio-adhesive gel formulations of the invention, i.e., the 0.15%, 0.45% and 0.6% testosterone by weight of the gel formulation, as reported in Examples 1-5, as compared to placebo;

[0078] FIG. 19 depicts Mean Corrected Free Testosterone Concentrations (Single-Dose Population);

[0079] FIG. 20 depicts Mean Corrected Total Testosterone Concentrations (Single-Dose Population);

[0080] FIG. 21 depicts Mean Corrected Dihydrotestosterone Concentrations (Single-Dose Population);

[0081] FIG. 22 depicts Mean Corrected Estradiol Concentrations (Single-Dose Population);

[0082] FIG. 23 depicts Mean Corrected SHBG Concentrations (Single-Dose Population);

[0083] FIG. 24 depicts Mean Observed Free Testosterone Concentrations (Single-Dose Population);

[0084] FIG. 25 depicts Mean Observed Total Testosterone Concentrations (Single-Dose Population);

[0085] FIG. 26 depicts Mean Observed Dihydrotestosterone Concentrations (Single-Dose Population);

[0086] FIG. 27 depicts Mean Observed Estradiol Concentrations (Single-Dose Population);

[0087] FIG. 28 depicts Mean Observed SHBG Concentrations (Single-Dose Population);

[0088] FIG. 29 depicts Mean Free Testosterone Plasma Concentrations (Multi-Dose Population);

[0089] FIG. 30 depicts Mean Total Testosterone Plasma Concentrations (Multi-Dose Population);

[0090] FIG. 31 depicts Mean Dihydrotestosterone Plasma Concentrations (Multi-Dose Population);

[0091] FIG. 32 depicts Mean Estradiol Plasma Concentrations (Multi-Dose Population);

[0092] FIG. 33 depicts Mean SHBG Plasma Concentrations (Multi-Dose Population);

[0093] FIG. 34 depicts Spaghetti Concentration Plots with Mean for Free Testosterone Plasma Concentrations (Multi-Dose Population);

[0094] FIG. 35 depicts Spaghetti Concentration Plots with Mean for Total Testosterone Plasma Concentrations (Multi-Dose Population);

[0095] FIG. 36 depicts Spaghetti Concentration Plots with Mean for Dihydrotestosterone Plasma Concentrations (Multi-Dose Population);

[0096] FIG. 37 depicts Spaghetti Concentration Plots with Mean for Estradiol Plasma Concentrations (Multi-Dose Population);

[0097] FIG. 38 depicts Spaghetti Concentration Plots with Mean for SHBG Plasma Concentrations (Multi-Dose Population); and

[0098] FIG. 39 depicts an intranasal applicator contemplated by and used in accordance with the present invention.

[0099] FIG. 40 depicts a manufacturing process for the testosterone gel formulations of the invention.

DETAILED DESCRIPTION

[0100] By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description and examples are given concerning the novel lower dosage strength intranasal testosterone gels, application devices and methods of the present invention.

I. DEFINITIONS

[0101] As used in the description of the invention and the appended claims, the singular forms “a”, “an” and “the” are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0102] As used herein, “at least one” is intended to mean “one or more” of the listed elements.

[0103] Singular word forms are intended to include plural word forms and are likewise used herein interchangeably where appropriate and fall within each meaning, unless expressly stated otherwise.

[0104] Except where noted otherwise, capitalized and non-capitalized forms of all terms fall within each meaning.

[0105] Unless otherwise indicated, it is to be understood that all numbers expressing quantities, ratios, and numerical properties of ingredients, reaction conditions, and so forth used in the specification and claims are contemplated to be able to be modified in all instances by the term “about”.

[0106] All parts, percentages, ratios, etc. herein are by weight unless indicated otherwise.

[0107] As used herein, “bioequivalence” or “bioequivalent”, refers to nasally administered testosterone gel formulations or drug products which are pharmaceutically equivalent and their bioavailabilities (rate and extent of absorption) after administration in the same molar dosage or amount are similar to such a degree that their therapeutic effects, as to safety and efficacy, are essentially the same. In other words, bioequivalence or bioequivalent means the absence of a significant difference in the rate and extent to which testosterone becomes available from such formulations at the site of testosterone action when administered at the same molar dose under similar conditions, e.g., the rate at which testosterone can leave such a formulation and the rate at which testosterone can be absorbed and/or become available at the site of action to affect anorgasmia and/or HSDD. In other words, there is a high degree of similarity in the bioavailabilities of two testosterone gel formulation pharmaceutical products for nasal administration (of the same galenic form) from the same molar dose, that are unlikely to produce clinically relevant differences in therapeutic effects, or adverse reactions, or both. The terms “bioequivalence”, as well as “pharmaceutical equivalence” and “therapeutic equivalence” are also used herein as defined and/or used by (a) the FDA, (b) the Code of Federal Regulations (“C.F.R.”), Title 21, (c) Health Canada, (d) European Medicines Agency (EMA), and/or (e) the Japanese Ministry of Health and Welfare. Thus, it should be understood that the present invention contemplates testosterone gel formulations for nasal administration or drug products that may be bioequivalent to other testosterone gel formulations for nasal administration or drug products of the present invention. By way of example, a first testosterone gel formulation for nasal administration or drug product is bioequivalent to a second testosterone gel formulation for nasal administration or drug product, in accordance with the present invention, when the measurement of at least one pharmacokinetic parameter(s), such as a C_{max}, T_{max}, AUC, etc., of the first testosterone gel formulation for nasal administration or drug product varies by no more than about $\pm 25\%$, when compared to the measurement of the same pharmacokinetic parameter for the second testosterone gel formulation for nasal administration or drug product of the present invention.

[0108] As used herein, “bioavailability” or “bioavailable”, means generally the rate and extent of absorption of testosterone into the systemic circulation and, more specifically, the rate or measurements intended to reflect the rate and extent to which testosterone becomes available at the site of action or is absorbed from a drug product and becomes available at the site of action. In other words, and by way of example, the extent and rate of testosterone absorption from a lower dosage strength gel formulation for nasal administration of the present invention as reflected by a time-concentration curve of testosterone in systemic circulation.

[0109] As used herein, the terms “pharmaceutical equivalence” or “pharmaceutically equivalent”, refer to testosterone gel formulations for nasal administration or drug products of the present invention that contain the same amount of testosterone, in the same dosage forms, but not necessarily containing the same inactive ingredients, for the same route of administration and meeting the same or comparable compendial or other applicable standards of identity, strength, quality, and purity, including potency and, where applicable, content

uniformity and/or stability. Thus, it should be understood that the present invention contemplates testosterone gel formulations for nasal administration or drug products that may be pharmaceutically equivalent to other testosterone gel formulations for nasal administration or drug products used in accordance with the present invention.

[0110] As used herein, “therapeutic equivalence” or “therapeutically equivalent”, means those testosterone gel formulations for nasal administration or drug products which (a) will produce the same clinical effect and safety profile when utilizing testosterone drug product to treat anorgasmia or HSDD in accordance with the present invention and (b) are pharmaceutical equivalents, e.g., they contain testosterone in the same dosage form, they have the same route of administration; and they have the same testosterone strength. In other words, therapeutic equivalence means that a chemical equivalent of a lower dosage strength testosterone formulation of the present invention (i.e., containing the same amount of testosterone in the same dosage form when administered to the same individuals in the same dosage regimen) will provide essentially the same efficacy and toxicity.

[0111] As used herein a “testosterone gel formulation for nasal administration” means a formulation comprising testosterone in combination with a solvent, a wetting agent, and a viscosity increasing agent.

[0112] As used herein, “increases” as it refers to the plasma testosterone level, also means that the plasma testosterone level is 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 500, 1000 or 10,000-fold or more greater in a subject that has been treated with a testosterone gel formulation for nasal administration of the invention as compared to the plasma testosterone level in the subject prior to treatment.

[0113] As used herein, “plasma testosterone level” means the level of testosterone in the plasma of a subject. The plasma testosterone level is determined by methods known in the art.

[0114] “Diagnosis” or “prognosis,” as used herein, refers to the use of information (e.g., biological or chemical information from biological samples, signs and symptoms, physical exam findings, psychological exam findings, etc.) to anticipate the most likely outcomes, timeframes, and/or responses to a particular treatment for a given disease, disorder, or condition, based on comparisons with a plurality of individuals sharing symptoms, signs, family histories, or other data relevant to consideration of a patient’s health status, or the confirmation of a subject’s affliction, e.g., with anorgasmia and/or HSDD.

[0115] A “subject” according to some embodiments is an individual whose signs and symptoms, physical exams findings and/or psychological exam findings are to be determined and recorded in conjunction with the individual’s condition (i.e., disease or disorder status) and/or response to a candidate drug or treatment.

[0116] “Subject,” as used herein, is preferably, but not necessarily limited to, a human subject. The subject may be male or female, and is preferably female, and may be of any race or ethnicity, including, but not limited to, Caucasian, African-American, African, Asian, Hispanic, Indian, etc. Subject as used herein may also include an animal, particularly a mammal such as a canine, feline, bovine, caprine, equine, ovine, porcine, rodent (e.g., a rat and mouse), a lagomorph, a primate (including non-human primate), etc., that may be treated in accordance with the methods of the present invention or screened for veterinary medicine or pharmaceutical drug development purposes. A subject according to some embodi-

ments of the present invention include a patient, human or otherwise, in need of therapeutic treatment for anorgasmia and/or HSDD.

[0117] “Treatment,” as used herein, includes any drug, drug product, method, procedure, lifestyle change, or other adjustment introduced in attempt to effect a change in a particular aspect of a subject’s health (i.e., directed to a particular disease, disorder, or condition).

[0118] “Drug” or “drug substance,” as used herein, refers to an active ingredient, such as a chemical entity or biological entity, or combinations of chemical entities and/or biological entities, suitable to be administered to a subject to (a) treat anorgasmia and/or (b) treat HSDD. In accordance with the present invention, the drug or drug substance is testosterone or a pharmaceutically acceptable salt or ester thereof.

[0119] The term “drug product,” as used herein, is synonymous with the terms “medicine,” “medicament,” “therapeutic intervention,” or “pharmaceutical product.” Most preferably, a drug product is approved by a government agency for use in accordance with the methods of the present invention. A drug product, in accordance with the present invention, is an intranasal gel formulated with a drug substance, i.e., testosterone.

[0120] “Disease,” “disorder,” and “condition” are commonly recognized in the art and designate the presence of signs and/or symptoms in an individual or patient that are generally recognized as abnormal and/or undesirable. Diseases or conditions may be diagnosed and categorized based on pathological changes. The disease or condition may be selected from the types of diseases listed in standard texts, such as Harrison’s Principles of Internal Medicine, 1997, or Robbins Pathologic Basis of Disease, 1998.

[0121] As used herein, “diagnosing” or “identifying a patient or subject having anorgasmia or HSDD” refers to a process of determining if an individual is afflicted with anorgasmia or HSDD.

[0122] As used herein, “control subject” means a subject that has not been diagnosed with anorgasmia and/or HSDD and/or does not exhibit any detectable symptoms associated with these diseases. A “control subject” also means a subject that is not at risk of developing anorgasmia and/or HSDD, as defined herein.

II. DISEASES

[0123] Anorgasmia

[0124] Anorgasmia is a type of sexual dysfunction in which a person cannot regularly achieve orgasm, even with adequate stimulation. In males the condition is often related to delayed ejaculation. Anorgasmia can often cause sexual frustration. Anorgasmia is far more common in females than in males and is especially rare in younger men. Anorgasmia is a very common occurrence in women, affecting 1 in 5 women worldwide.

[0125] The condition is sometimes classified as a psychiatric disorder. However, it can also be caused by medical problems such as diabetic neuropathy, multiple sclerosis, genital mutilation, complications from genital surgery, pelvic trauma (such as from a straddle injury caused by falling on the bars of a climbing frame, bicycle or gymnastics beam), hormonal imbalances, total hysterectomy, spinal cord injury, cauda equina syndrome, uterine embolisation, childbirth trauma (vaginal tearing through the use of forceps or suction or a large or unclosed episiotomy), vulvodynia and cardiovascular disease.

[0126] A comprehensive definition of female orgasm, as Meston et al. proposed, is as follows:

[0127] “[A] variable, transient peak sensation of intense pleasure creating an altered state of consciousness, usually accompanied by involuntary, rhythmic contractions of the pelvic striated circumvaginal musculature, often with concomitant uterine and anal contractions and myotonia that resolves the sexually-induced vasocongestion (sometimes only partially), usually with an induction of well-being and contentment.”

[0128] The Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) defines female orgasmic disorder (FOD, formerly inhibited female orgasm) as a persistent or recurrent delay in, or absence of, orgasm following a normal sexual excitement phase.

[0129] The type or intensity of stimulation that triggers female orgasm varies widely among women. Therefore, the diagnosis of female orgasmic disorder, according to the DSM-IV-TR, is based on these 3 criteria:

[0130] Criterion A: A clinician must judge that a woman’s orgasmic capacity is less than what is reasonable for her age, sexual experience, and the adequacy of sexual stimulation she receives.

[0131] Criterion B: The disturbance must cause marked distress or interpersonal difficulty.

[0132] Criterion C: Another axis I disorder (except another sexual dysfunction) does not account for the orgasmic dysfunction better than female orgasmic disorder does, and the orgasmic dysfunction is not exclusively due to the direct physiologic effects of a substance (e.g., drug of abuse, medication) or a general medical condition.

[0133] In the DSM-IV-TR, female orgasmic disorder specifiers include the following:

[0134] Lifelong or acquired

[0135] Generalized or situational

[0136] Due to psychological or combined factors

[0137] The presence of a normal sexual excitement phase is a prerequisite for female orgasmic disorder. In other words, if the absence of orgasm follows a time of decreased desire for sexual activity, an aversion to genital sexual contact, or a decreased lubrication-swelling response, diagnoses such as hypoactive sexual desire disorder, sexual aversion disorder, or female sexual arousal disorder, respectively, might be more appropriate, even if anorgasmia is the common final outcome.

[0138] A wide range of physical and psychological causes of anorgasmia have been identified.

[0139] They include the following:

[0140] A history of sexual abuse or rape

[0141] Boredom and monotony in sexual activity

[0142] Certain prescription drugs, including fluoxetine (Prozac), paroxetine (Paxil), and sertraline (Zoloft)

[0143] Hormonal disorders, hormonal changes due to menopause, and chronic illnesses that affect general health and sexual interest

[0144] Medical conditions that affect the nerve supply to the pelvis (such as multiple sclerosis, diabetic neuropathy, and spinal cord injury)

[0145] Negative attitudes toward sex (usually learned in childhood or adolescence)

[0146] Shyness or embarrassment about asking for whatever type of stimulation works best

[0147] Strife or lack of emotional closeness within the relationship

Physical Causes

[0148] A wide range of illnesses, physical changes and medications can interfere with orgasm:

[0149] Medical diseases. Any illness can affect this part of an individual’s sexuality, including diabetes and neurological diseases, such as multiple sclerosis. Orgasm may also be affected by gynecologic surgeries, such as hysterectomy or cancer surgeries. In addition, lack of orgasm often goes hand in hand with other sexual problems, such as painful intercourse.

[0150] Medications. Many prescription and over-the-counter medications can interfere with orgasm. This includes blood pressure medications, antihistamines and antidepressants—particularly selective serotonin reuptake inhibitors (SSRIs). In men, SSRIs can actually result in both anorgasmia and inability to obtain an adequate erection for satisfactory sexual activity (erectile dysfunction).

[0151] Alcohol and drugs. A glass of wine may make you feel amorous, but too much alcohol can cramp your ability to climax; the same is true of street drugs.

[0152] The aging process. As you age, normal changes in your anatomy, hormones, neurological system and circulatory system can affect your sexuality. The drop in estrogen that occurs during the transition to menopause can be a particularly notable foe of orgasm. Lower levels of this female hormone can decrease sensations in the clitoris, nipples and skin and impede blood flow to the vagina and clitoris, which can delay or stop orgasm entirely. Still, anorgasmia isn’t limited to older women. And many women say sex becomes more satisfying with age.

Psychological Causes

[0153] Many psychological factors play a role in your ability to orgasm, including:

[0154] Mental health problems, such as anxiety or depression

[0155] Performance anxiety

[0156] Stress and financial pressures

[0157] Cultural and religious beliefs

[0158] Fear of pregnancy or sexually transmitted diseases

[0159] Embarrassment

[0160] Guilt about enjoying sexual experiences

Relationship Issues

[0161] Many couples who are experiencing problems outside of the bedroom will also experience problems in the bedroom. These overarching issues may include:

[0162] Lack of connection with your partner

[0163] Unresolved conflicts or fights

[0164] Poor communication of sexual needs and preferences

[0165] Infidelity or breach of trust

[0166] A common cause of situational anorgasmia, in both men and women, is the use of anti-depressants, particularly selective serotonin reuptake inhibitors (SSRIs). Post-SSRI sexual dysfunction (PSSD) is a name given to a reported iatrogenic sexual dysfunction caused by the previous use of SSRI antidepressants. Though reporting of anorgasmia as a side effect of SSRIs is not precise, it is estimated that 15-50% of users of such medications are affected by this condition. The chemical amantadine has been shown to relieve SSRI-induced anorgasmia in some, but not all, people.

[0167] Another cause of anorgasmia is opiate addiction, particularly to heroin.

[0168] About 15% of women report difficulties with orgasm, and as many as 10% of women in the United States have never climaxed. Many women who orgasm regularly only climax about 50-70% of the time.

[0169] The major symptoms of anorgasmia are inability to experience orgasm or long delays in reaching orgasm. Different types of anorgasmia have been identified.

Primary Anorgasmia

[0170] Primary anorgasmia is a condition where one has never experienced an orgasm. This is significantly more common in women, although it can occur in men who lack the gladiopudendal (bulbocavernosus) reflex.

[0171] Women with this condition can sometimes achieve a relatively low level of sexual excitement. Frustration, restlessness, and pelvic pain or a heavy pelvic sensation may occur because of vascular engorgement.

[0172] On occasion, there may be no obvious reason why orgasm is unobtainable. In such cases, women report that they are unable to orgasm even if they have a caring, skilled partner, adequate time and privacy, and an absence of medical issues which would affect sexual satisfaction. It should be noted that the attention and skill of one's partner are not inextricably linked to woman's internal, implicit comfort level. Thus, anorgasmia in a woman whose partner is adequately attentive and skilled should not be regarded as a clinical mystery.

[0173] Some social theorists believe that inability to orgasm may be related to residual psychosocial perceptions that female sexual desire is somehow 'wrong,' and that this stems from the age of Victorian repression. It is thought that this view may impede some women—perhaps those raised in a more repressed environment—from being able to experience natural and healthy sexual feeling. While such proposals may have a place in academic social theory, they have not been established scientifically. Therefore, an idea such as this may be a component of treatment as one consideration among many, but responsible clinical practice should not be guided, based on, or informed by it.

[0174] Primary male anorgasmia is more common among circumcised men than intact men. As many men age, they lose the ability to orgasm even if they still ejaculate. The pleasure of orgasm may diminish in older men.

Secondary Anorgasmia

[0175] Secondary anorgasmia is the loss of the ability to have orgasms. Particularly, you used to have orgasms, but now experience difficulty reaching climax. The cause may be alcoholism, depression, grief, pelvic surgery (such as total hysterectomy) or injuries, certain medications, illness, estrogen deprivation associated with menopause or an event that has violated the patient's sexual value system.

[0176] Secondary anorgasmia is close to 50% among males undergoing prostatectomy; 80% among radical prostatectomies. This is a serious adverse result because radical prostatectomies are usually given to younger males who are expected to live more than 10 years. At more advanced ages, the prostate is more unlikely to grow during that person's remaining lifetime. This is generally caused by damage to the primary nerves serving the penile area, which pass near the prostate gland. Removal of the prostate frequently damages

or even completely removes these nerves, making sexual response unreasonably difficult.

[0177] Due to the existence of these nerves in the prostate, surgeons performing sex reassignment surgery on transsexual male to female patients avoid removing the prostate. This leaves the nerves that will then lead to the newly-formed clitoris, and decreases the chances that the patient will not respond to clitoral stimulation after surgery. Additionally, by leaving the prostate in the patient, the surgeon allows it to be situated close to the wall of the newly-formed vagina, which may potentially increase stimulation during vaginal intercourse after the procedure.

Situational Anorgasmia

[0178] People who are orgasmic in some situations may not be in others. This means that you are able to orgasm only during certain circumstances, such as during oral sex or masturbation. This is very common in women. In fact, about 80% of women experience orgasm from stimulation of the clitoris.

[0179] A person may have an orgasm from one type of stimulation but not from another, a person may achieve orgasm with one partner but not another, or have an orgasm only under certain conditions or only with a certain type or amount of foreplay. These common variations are within the range of normal sexual expression and should not be considered problematic.

[0180] Factors that may affect whether or not an individual is orgasmic, include fatigue, emotional concerns, feeling pressured to have sex when he or she is not interested, or a partner's sexual dysfunction. In the relatively common case of female situational anorgasmia during penile-vaginal intercourse, some sex therapists recommend that couples incorporate manual or vibrator stimulation during intercourse, or using the female-above position as it may allow for greater stimulation of the clitoris by the penis or pubic symphysis or both, and it allows the woman better control of movement.

[0181] General Anorgasmia

[0182] This means an individual cannot orgasm in any situation with any partner.

Random Anorgasmia

[0183] Some people are orgasmic but not in enough instances to satisfy their sense of what is appropriate or desirable.

Prevention

[0184] A healthy attitude toward sex, and education about sexual stimulation and response will minimize problems.

[0185] Couples who clearly communicate their sexual needs and desires, verbally or nonverbally, will experience orgasmic dysfunction less frequently.

[0186] It is also important to realize that sexual response is a complex coordination of the mind and the body, and both need to be functioning well for orgasms to happen.

Symptoms

[0187] The symptom of orgasmic dysfunction is being unable to reach orgasm, taking longer than you want to reach orgasm, or having only unsatisfying orgasms.

Signs and Tests

[0188] A complete medical history and physical examination needs to be done, but results are almost always normal. If the problem began after starting a medication, this should be discussed with the doctor who prescribed the drug. A qualified specialist in sex therapy may be helpful.

Treatment

[0189] Treatment can involve education, cognitive behavioral therapy, teaching orgasm by focusing on pleasurable stimulation, and directed masturbation.

[0190] Most women require clitoral stimulation to reach an orgasm. Incorporating this into sexual activity may be all that is necessary. If this doesn't solve the problem, then teaching the woman to masturbate may help her understand what she needs to become sexually excited.

[0191] A series of couple exercises to practice communication, more effective stimulation, and playfulness can help. If relationship difficulties play a role, treatment may include communication training and relationship enhancement work.

[0192] Medical problems, new medications, or untreated depression may need evaluation and treatment in order for orgasmic dysfunction to improve.

[0193] If other sexual dysfunctions (such as lack of interest and pain during intercourse) are happening at the same time, these need to be addressed as part of the treatment plan.

[0194] HSDD

[0195] Hypoactive sexual desire disorder (HSDD), is considered as a sexual dysfunction and is listed under the Sexual and Gender Identity Disorders of the DSM-IV. It was first included in the DSM-III under the name Inhibited Sexual Desire Disorder, but the name was changed in the DSM-III-R.

[0196] HSDD is characterized as a lack or absence of sexual fantasies and desire for sexual activity for some period of time. For this to be regarded as a disorder, it must cause marked distress or interpersonal difficulties and not be better accounted for by another mental disorder (i.e. depression), a drug (legal or illegal), or some other medical condition.

[0197] There are various subtypes. HSDD can be general (general lack of sexual desire) or situational (still has sexual desire, but lacks sexual desire for current partner), and it can be acquired (HSDD started after a period of normal sexual functioning) or life-long (the person has always had no/low sexual desire.)

[0198] In the early versions of the DSM, there were only two sexual dysfunctions listed: frigidity (for women) and impotence (for men).

[0199] In 1970, Masters and Johnson published their book *Human Sexual Inadequacy* describing sexual dysfunctions, though these included only dysfunctions dealing with the function of genitals such as premature ejaculation and impotence for men, and anorgasmia and vaginismus for women. Prior to Masters and Johnson's research, female orgasm was assumed to originate primarily from vaginal, rather than clitoral, stimulation. Consequently, feminists have argued that "frigidity" was "defined by men as the failure of women to have vaginal orgasms".

[0200] Following this book, sex therapy increased throughout the 1970s. Reports from sex-therapists about people with low sexual desire are reported from at least 1972, but labeling this as a specific disorder did not occur until 1977. In that year, sex therapists Helen Singer Kaplan and Harold Lief independently of each other proposed creating a specific category for

people with low or no sexual desire. Lief named it "Inhibited Sexual Desire," and Kaplan named it "Hypoactive Sexual Desire." The primary motivation for this was that previous models for sex therapy assumed certain levels of sexual interest in one's partner and that problems were only caused by abnormal functioning/non-functioning of the genitals or performance anxiety but that therapies based on those problems were ineffective for people who did not sexually desire their partner. The following year, 1978, Lief and Kaplan together made a proposal to the APA's taskforce for sexual disorders for the DSM III, of which Kaplan and Lief were both members. The diagnosis of Inhibited Sexual Desire (ISD) was added to the DSM when the 3rd edition was published in 1980.

[0201] For understanding this diagnosis, it is important to recognize the social context in which it was created. In some cultures, low sexual desire may be considered normal and high sexual desire is problematic. In others, this may be reversed. Some cultures try hard to restrain sexual desire. Others try to excite it. Concepts of "normal" levels of sexual desire are culturally dependent and rarely value-neutral. In the 1970s, there were strong cultural messages that sex is good for you and "the more the better." Within this context, people who were habitually uninterested in sex, who in previous times may not have seen this as a problem, were more likely to feel that this was a situation that needed to be fixed. They may have felt alienated by dominant messages about sexuality and increasingly people went to sex-therapists complaining of low sexual desire. It was within this context that the diagnosis of ISD was created.

[0202] In the revision of the DSM-III, published in 1987 (DSM-III-R), ISD was subdivided into two categories: Hypoactive Sexual Desire Disorder and Sexual Aversion Disorder (SAD). The former is a lack of interest in sex and the latter is a phobic aversion to sex. In addition to this subdivision, one reason for the change is that the committee involved in revising the psychosexual disorders for the DMS-III-R thought that term "inhibited" suggests psychodynamic etiology (i.e. that the conditions for sexual desire are present, but the person is, for some reason, inhibiting their own sexual interest.) The term "hypoactive sexual desire" is more awkward, but more neutral with respect to the cause. The DSM-III-R estimated that about 20% of the population had HSDD. In the DSM-IV (1994), the criterion that the diagnosis requires "marked distress or interpersonal difficulty" was added.

Causes

[0203] Low sexual desire is not equivalent to HSDD because of the requirement that the low sexual desire causes marked distress and interpersonal difficulty and because of the requirement that the low desire is not better accounted for by another disorder in the DSM or by a general medical problem, so it is difficult to say exactly what causes HSDD. It is easier to describe, instead, what causes low sexual desire.

[0204] In men, there are theoretically more types of HSDD/low sexual desire, typically men are only diagnosed with one of three subtypes.

[0205] Lifelong/Generalized:

[0206] The man has little or no desire for sexual stimulation (with a partner or alone) and never has.

[0207] Acquired/Situational:

[0208] The man was previously sexually interested in his present partner but now lacks sexual interest in them but has desire for sexual stimulation (i.e. alone or with someone other than his present partner.)

[0209] Acquired/Generalized:

[0210] The man previously had sexual interest in his present partner, but lacks interest in sexual activity, partnered or solitary.

[0211] Though it can sometimes be difficult to distinguish between these types, they do not necessarily have the same etiology. The cause of lifelong/generalized HSDD is unknown. In the case of acquired/generalized low sexual desire, possible causes include various medical/health problems, psychiatric problems, low levels of testosterone or high levels of prolactin. One theory suggests that sexual desire is controlled by a balance between inhibitory and excitatory factors. This is thought to be expressed via neurotransmitters in selective brain areas. A decrease in sexual desire may therefore be due to an imbalance between neurotransmitters with excitatory activity like dopamine and norepinephrine and neurotransmitters with inhibitory activity, like serotonin. Low sexual desire can also be a side effect of various medications. In the case of acquired/situational HSDD, possible causes include intimacy difficulty, relationship problems, sexual addiction, and chronic illness of the man's partner. The evidence for these is somewhat in question. Some claimed causes of low sexual desire are based on empirical evidence. However, some are based merely on clinical observation. In many cases, the cause of HSDD is simply unknown.

[0212] There are some factors that are believed to be possible causes of HSDD in women. As with men, various medical problems, psychiatric problems (such as mood disorders), or increased amounts of prolactin can cause HSDD. Other hormones are believed to be involved as well. Additionally, factors such as relationship problems or stress are believed to be possible causes of reduced sexual desire in women.

III. TESTOSTERONE

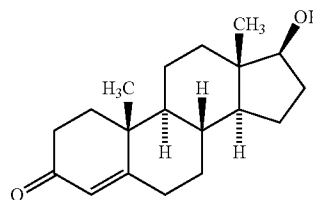
[0213] The steroid hormone testosterone is the active ingredient in the testosterone gel formulations of the invention. The manufacture of the drug substance presents no potential risk for humans; the synthesis route is well-characterized.

TABLE 1

Nomenclature Testosterone	
INN name	Testosterone
Compendial name	Testosterone
Chemical name	17 β -Hydroxyandrost-4-en-3-one
Other non-proprietary names	Androst-4-en-3-one, 17-hydroxy-, (17 β)- Trans-testosterone Δ^4 -androst-17 β -ol-3-one
CAS registry number	58-22-0
Proquina code	8139

Structural Formula

[0214]



Molecular Formula

[0215]



Relative Molecular Mass

[0216] 288.4

[0217] The physical chemical properties of testosterone are listed in Table 2.

TABLE 2

General Properties of Testosterone	
Appearance	White or slightly creamy white crystals or crystalline powder. It is odourless, and stable in air.
Solubility	Practically insoluble in water (0.024 g/L), freely soluble in dehydrated alcohol, chloroform and in methylene chloride, soluble in dioxane and in vegetable oils; slightly soluble in ether.
Melting range	153° C. to 157° C.
Specific rotation	+101° to +105° (dioxane)
Loss on drying	Not more than 1.0%
UV max	238 nm
Storage	Protected from light

[0218] Testosterone, for testosterone gel formulations of the invention, appears as white or slightly creamy white crystals or crystalline powder. It is freely soluble in methanol and ethanol, soluble in acetone and isopropanol and insoluble in n-heptane. It can also be considered as insoluble in water ($S_{20^\circ C.} = 2.41 \times 10^{-2} \text{ g/L} \pm 0.04 \times 10^{-2} \text{ g/L}$); its n-Octanol/Water partition coefficient ($\log P_{OW}$ determined by HPLC) is 2.84. The solubility of testosterone in oils was determined to be 0.8% in isopropylmyristate, 0.5% in peanut oil, 0.6% in soybean oil, 0.5% in corn oil, 0.7% in cottonseed oil and up to 4% in castor oil.

[0219] Because testosterone is fully dissolved within the formulations of the present invention, physical characteristics of the drug substance do not influence the performance of the drug product, testosterone gel formulations of the invention. The manufacturability of testosterone gel formulations of the invention, however is influenced by the particle size of testosterone. When using a particle size of $50\% \leq 25$ microns, $90\% \leq 50$ microns the solubility of the drug substance in the matrix is especially favorable.

[0220] In accordance with the present invention, the testosterone drug can be in, for instance, crystalline, amorphous, micronized, non-micronized, powder, small particle or large particle form when formulating to intranasal testosterone gels of the present invention. An Exemplary range of testosterone particle sizes include from about 0.5 microns to about 200 microns. Preferably, the testosterone particle size is in a range

of from about 5 microns to about 100 microns, and the testosterone is in crystalline or amorphous and non-micronized or micronized form. Preferably, the testosterone is in crystalline or amorphous micronized form.

[0221] The molecular structure of testosterone contains no functional groups that can be protonated or deprotonated in the physiological pH-range. Therefore testosterone is to be considered as a neutral molecule with no pKa value in the range 1-14. Because it is neutral, testosterone is compatible with excipients.

Drug Product

[0222] The testosterone gel formulations of the invention are viscous and thixotropic, oil-based formulations containing a solution of testosterone intended for intranasal application. The non-irritating formulation is designed to adhere to the inner nose. In addition, it acts as a controlling matrix, thus allowing sustained drug delivery through the nasal mucosa.

[0223] Other pharmacologically inactive ingredients in the testosterone intranasal gel are castor oil USP, oleoyl macroglycerides EP and colloidal silicon dioxide NF. None of these excipients are of human or animal origin. All excipients are well-known and listed in the "Inactive Ingredient" list for Approved Drug Products issued by the FDA.

[0224] According to the "Handbook of Pharmaceutical Additives" oleoyl polyoxyglycerides are used as hydrophilic oil for topicals, injectables and nasals. In FDA-approved medicinal products it is used as co-emulsifier in topical emulsions/lotions/creams and in vaginal emulsions/creams. In France this excipient is approved for nasal preparations such as "Rhino-Sulforgan" (Laboratoire Jolly-Jatel, France; containing 10% oleoyl polyoxyglycerides) and "Huile Gome-nolee 2%" ("Laboratoire Gomenol, France; containing 10% oleoyl polyoxyglycerides). Hence, like for castor oil it can be deduced that oleoyl polyoxyglycerides is suitable for an application route where safety and tolerability are of highest importance (e.g. injectables and nasal or vaginal preparations).

[0225] Oleoyl macroglycerides are also referred to as Labrafil M 1944 CS, apricot kernel oil PEG-6 esters, Peglicol-5-oleate, mixture of glycerides and polyethylene esters. The castor oil, which is used as a solvent for testosterone gel formulations of the invention is a fixed oil. Such oils have the advantage of being non-volatile or spreading (in contrast to essential oils or liquid paraffin), but have the disadvantage of being hydrophobic. The nasal mucosa contains 95-97% water. Without the oleoyl macroglycerides, the castor oil containing the active ingredient would form a non-interactive layer on the mucous membrane. In order to achieve adequate contact between the castor oil layer and the mucous membrane, the hydrophilic oleoyl macroglycerides oil is added to the formulation to form an emulsion between the castor oil and the mucosa fluid.

[0226] Oleoyl macroglycerides are used in semi-solids at concentrations ranging from about 3 to 20%, depending on

the application. The amount of oleoyl macroglycerides in testosterone gel formulations of the invention is high enough to allow for a better contact of the carrier oil with the mucous membrane and low enough to have minimal impact on the amount of testosterone that can be incorporated into the carrier oil. A favourable concentration of oleoyl macroglycerides in testosterone gel formulations of the invention is found to be 4% of the formulation.

[0227] According to the "Handbook of Pharmaceutical Additives" colloidal silicon dioxide is used as an oil adsorbent, thermal stabilizer and gellant. In FDA-approved medicinal products it is used in dental gels, sublingual tablets, endocervical gel, suppositories, vaginal emulsions/creams/tablets/tampons and capsules for inhalation. Furthermore, it is used as an excipient in "Testoderm with adhesives" (Alza Corporation, approved in 1996) a testosterone transdermal patch. Hence, it can be deduced that colloidal silicon dioxide is suitable for an application route where safety and tolerability are of highest importance (e.g. inhalations, endocervical, vaginal or rectal preparations).

[0228] For clinical trial supplies, testosterone intranasal gel is supplied in unit-dose syringes consisting of a syringe body made from polypropylene, a plunger molded from polyethylene and a syringe cap made from high density polyethylene. The syringes are wrapped in aluminum foil as secondary packaging. The content of a syringe (125 mg) amounts to 0.10 to 1.5 mg of testosterone.

[0229] The oil in testosterone gel formulations of the invention is thickened with colloidal silicon dioxide, which acts as a gel-forming agent. This compound is used commonly for stiffening oleogels.

[0230] The intended dosage form for testosterone gel formulations of the invention is a semi-solid, not a liquid. The formulation is thickened with colloidal silicon dioxide. It is believed that colloidal silicon dioxide contributes to the thixotropic properties of the gel, simplifying drug delivery to the nostril.

[0231] Colloidal silicon dioxide is generally an inert material which is well tolerated as an excipient in mucosal applications such as suppositories. Colloidal silicon dioxide is typically used in these preparations at concentrations ranging from about 0.5 to 10%. The concentration of colloidal silicon dioxide in testosterone gel formulations of the invention is high enough to achieve gel formation but at a level that has minimal impact on testosterone incorporation into the carrier oil.

[0232] Preferably, the intranasal testosterone gels of the present invention have in general, a viscosity in the range of between about 3,000 cps and about 27,000 cps. It should nevertheless be understood by those versed in this art that, while the above-mentioned viscosity range is believed to be a preferred viscosity range, any suitable viscosities or viscosity ranges that do not defeat the objectives of the present invention are contemplated.

[0233] A detailed description of batches of a testosterone gel formulation of the invention is shown in Table 3.

TABLE 3

Composition of a testosterone gel formulation of the invention						
Component	Amount (% w/w) 0.15%	Amount (% w/w) 0.24%	Amount (% w/w) 0.45%	Amount (% w/w) 0.48%	Amount (% w/w) 0.60%	Amount (% w/w) 0.72%
Testosterone	0.15%	0.15%	0.45%	0.48%	0.60%	0.72%
Castor oil	91.85%	91.76%	91.55%	91.52%	91.4%	91.28%
Oleoyl macroglycerides	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%
Colloidal silicon dioxide	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%

[0234] The testosterone gel formulations of the invention are stored at room temperature (20-25° C. or 68 to 77° F.). See also Example 11. Temperature excursions from 15 to 30° C. or 59 to 86° F. are permissible for the testosterone gel formulations of the inventions. The stability data available to date are conclusive to support a 24-month shelf life. Unit dose syringes are chosen for the primary packaging of the clinical materials for this clinical trial to allow for ease of dosing, ability to generate multiple doses by varying the fill volume and consistency of dose delivered. The syringe consists of a syringe body, a plunger and a syringe cap. The syringes body is molded from polypropylene, the plunger is molded from polyethylene and the cap is HDPE. These syringes are designed and manufactured to deliver sterile and non-sterile solutions, liquids and gels at low volumes. For additional protection from the environment (i.e., exposure to dirt, light, humidity and oxygen), the syringes are packed in a foil-laminate overwrap pouch.

[0235] The syringes and caps are designed for use in a clinical setting and meet the requirements of the EU Medical Devices Directive 93/42/EEC of Jun. 14, 1993 and as amended. As this container closure is only intended for use in this portion of the clinical program, no additional studies are performed on the syringe and syringe components. See also Example 11 and FIG. 39.

[0236] For a further element of protection, two syringes are contained in secondary packaging consisting of an aluminium foil pouch. Two syringes are packaged in the aluminium foil pouch and each pouch is sealed.

[0237] The pouch consists of a flexible, 3-layered-foil-laminate of a) polyester 12 micron, b) aluminum 12 micron and c) a polyethylene 75 micron. It is manufactured by Floeter Flexibles GmbH, and supplied under the name "CLIMA-PAC II 12-12-75".

[0238] INTRINSA®

[0239] Procter & Gamble developed a transdermal therapeutic system containing testosterone as active substance for the treatment of HSDD (SD Intrinsa®). Four controlled clinical studies were performed (2 in Phase II b, 2 in Phase III). The 300 µg testosterone transdermal system is effective in the treatment of HSDD in surgically menopausal women on concomitant estrogen therapy. The women who received testosterone experienced increased frequency of satisfying sexual activity, increased sexual desire, and decreased distress compared with women who received placebo. Improvements were also seen in all other efficacy endpoints (i.e., arousal, pleasure, orgasm, responsiveness, self-image, concerns). The testosterone serum levels were increased to the physiological range of premenopausal women, but did not exceed this range.

[0240] Overall, treatment benefits were seen as early as 4 weeks with maximal effects in total satisfying episodes and sexual desire seen by approximately 12 weeks. Beneficial effects were maintained for the remainder of the 24-week efficacy period. At week 24, trial results showed that Intrinsa® significantly increased the frequency of total satisfying episodes compared to placebo ($p<0.05$) and also experienced a significantly greater increase in sexual desire domain of PSFS (profile of female sexual function) and a significantly greater decrease in personal distress ($p<0.05$) in patients on placebo. Intrinsa® is approved for the treatment of HSDD in bilaterally oophorectomised and hysterectomised women receiving concomitant estrogen therapy in the European Union.

[0241] LIBIGEL®

[0242] Biosante developed a testosterone gel designed to be quickly absorbed through the skin after a once-daily application on the upper arm, delivering testosterone to the bloodstream evenly over time for the treatment of HSDD. One Phase II study has been performed and 2 Phase III studies are ongoing.

[0243] The Phase II trial results showed LibiGel significantly increased the number of satisfying sexual events by 238% versus baseline ($p<0.0001$); this increase also was significant versus placebo ($p<0.05$). In this study, the effective dose of LibiGel produced testosterone blood levels within the normal range for pre-menopausal women and had a safety profile similar to that observed in the placebo group. In addition, no serious adverse events and no discontinuations due to adverse events occurred in any subject receiving LibiGel.

IV. DOSAGES AND MODES OF ADMINISTRATION

[0244] The invention provides for gel formulations of testosterone to be administered intranasally, wherein the dosage of the formulation is from about 0.15% testosterone by weight of said gel formulation to about 0.6% testosterone by weight of said gel formulation, for example, 0.15% testosterone by weight of the gel formulation, 0.45% testosterone by weight of said gel formulation and 0.6% by weight of the gel formulation.

V. USES

[0245] The methods of the invention are used to treat anorgasmia and/or HSDD in a diagnosed with one or both of these conditions. The invention also provides for intranasal testosterone gel formulations that can be used to treat anorgasmia or HSDD in a patient diagnosed with one or both of these conditions.

[0246] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

[0247] Having now generally described the invention, the same will be more readily understood through reference to the following Examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

[0248] The following examples are put forth for illustrative purposes only and are not intended to limit the scope of what the inventors regard as their invention.

Example 1

Description and Composition of Testosterone Gel Formulations of the Invention

[0249] The compositions of three different concentrations of the drug product to be administered in this clinical trial are provided in the tables below.

Description of Dosage Form

[0250] The testosterone gel formulations of the invention are viscous and thixotropic, oil-based formulations containing solubilized testosterone intended for intranasal application. The drug product is formulated with the compendial inactive ingredients: castor oil, oleoyl polyoxyl-glycerides and colloidal silicon dioxide.

[0251] Three different doses of the testosterone gel formulations of the invention are intranasally administered: 0.15% w/w, 0.45% w/w and 0.6% w/w. An overage is added to each syringe to account for the gel that is retained in the syringe after dosing. This overage remains consistent at 23 μ l, regardless of volume of gel in the syringe.

Lower Dosage Strength Intranasal Testosterone Compositions

[0252]

TABLE 4

Components, Quantity, Quality Standards and Function - 0.15% testosterone gel formulation of the invention					
Component	Amount (% w/w)	Amount per Syringe (mg)	Amount Delivered per Dose (mg)	Function	Quality Standard
Testosterone	0.15%	0.18	0.15	Active ingredient	USP
Castor oil	91.85%	112.98	91.85	Solvent	USP
Oleoyl polyoxyl-glycerides	4.0%	4.92	4.0	Wetting agent (hydrophilic oil)	Ph. Eur./NF
Colloidal silicon dioxide	4.0%	4.92	4.0	Viscosity increasing agent	NF
Total	100%	123 mg	100 mg		

TABLE 5

Components, Quantity, Quality Standards and Function - 0.45% testosterone gel formulation of the invention					
Component	Amount (% w/w)	Amount per Syringe (mg)	Amount Delivered per Dose (mg)	Function	Quality Standard
Testosterone	0.45%	0.55	0.45	Active ingredient	USP
Castor oil	91.55%	112.61	91.55	Solvent	USP
Oleoyl polyoxyl-glycerides	4.0%	4.92	4.0	Wetting agent (hydrophilic oil)	Ph. Eur./NF
Colloidal silicon dioxide	4.0%	4.92	4.0	Viscosity increasing agent	NF
Total	100%	123 mg	100 mg		

TABLE 6

Components, Quantity, Quality Standards and Function - 0.6% testosterone gel formulation of the invention					
Component	Amount (% w/w)	Amount per Syringe (mg)	Amount Delivered per Dose (mg)	Function	Quality Standard
Testosterone	0.6%	0.74	0.6	Active ingredient	USP
Castor oil	91.4%	112.42	91.4	Solvent	USP
Oleoyl polyoxyl-glycerides	4.0%	4.92	4.0	Wetting agent (hydrophilic oil)	Ph. Eur./NF
Colloidal silicon dioxide	4.0%	4.92	4.0	Viscosity increasing agent	NF
Total	100%	123 mg	100 mg		

Container

[0253] Testosterone gel formulations of the invention are supplied in unit-dose polypropylene syringes. Two syringes of each dosage are packaged in a protective aluminium foil pouch.

Example 2

Intranasal Testosterone Gel Formulations

[0254] The testosterone gel formulations of the invention, are formulations of testosterone in an intranasal gel proposed for assessing the pharmacokinetic and pharmacodynamics of three different doses of testosterone gel formulations of the invention, compared to Intrinsa® and placebo for testosterone gel formulations of the invention in women with hypoactive sexual desire disorder (HSDD) and secondary anorgasmia (SA).

[0255] The active ingredient, testosterone, is sourced from Bayer Schering. Challenges for nasal delivery include:

[0256] requirements for larger particles than pulmonary administration (i.e., only particles >10 μ m are sufficiently heavy to avoid entering the respiratory tract);

[0257] concentrations must be higher due to the smaller volumes that can be administered;

[0258] rapid clearance of the therapeutic agent from the site of deposition results in a shorter time available for absorption;

[0259] potential for local tissue irritation; and

[0260] limited formulation manipulation possibilities to alter drug delivery profiles.

[0261] Testosterone is indicated for the treatment of HSDD in bilaterally oophorectomised and hysterectomised (surgically induced menopausal) women receiving concomitant estrogen therapy. It is also indicated for the treatment of hormone replacement therapy in the treatment of hypogonadism in men. The currently available options for administration of testosterone are oral, buccal, injectable, implantable and transdermal.

[0262] An intranasal testosterone (3.2%) gel, TBS-1 gel, is developed for the treatment of hypogonadism in men and has been administered to hypogonadal men in several clinical trials (Mattern, C. et al., 2008 The Aging Male 11(4):171-178

(December 2008), which is incorporated herein by reference in its entirety). The intranasal testosterone gel for women, testosterone gel formulations of the present invention, are developed at concentrations ranging from about 0.15% to about 0.6% testosterone.

Example 3

Overages

Testosterone Gel Formulations of the Invention

[0263] No overage is added to the formulation. An overage is added to each syringe to account for the gel that is retained in the syringe after dosing. This overage remains consistent at 23 regardless of volume of gel in the syringe. The theoretical fill and dispensed amounts for testosterone gel formulations of the invention are provided below.

Syringe Dosage	Theoretical Fill Volume (μl)	Theoretical Dispensed Volume (μl)
0.15% Testosterone Gel formulation of the Invention	123	100
0.45% Testosterone Gel formulation of the Invention	123	100
0.6% Testosterone Gel formulation of the Invention	123	100

Example 4

Physicochemical and Biological Properties

Testosterone Gel Formulations of the Invention

[0264] The testosterone gel formulations of the invention has a viscosity in the range of 3,000 to 10,000 mPa·sec. The viscosity is important because it facilitates maintenance of the gel in the nasal cavity in contact with the nasal mucosa. When the viscosity is less than approximately 3,000 mPa·sec (i.e., 3,000 centipoise), the gel tends to be drawn by gravity out of the nasal cavity.

Example 5

Batch Formula

Testosterone Gel Formulations of the Invention

[0265] Three different concentrations of testosterone gel formulations of the invention, 0.15%, 0.45% and 0.6%, are manufactured for the proposed clinical trial. The batch formulae for these batches are presented in Table 5 below.

TABLE 5

Batch Formulae for 0.15%, 0.45% and 0.6% testosterone gel formulations of the invention at the 8 kg Batch Size			
Components	Quantity per Batch (g)		
	0.15%	0.45%	0.6%
Testosterone, USP	12	36	48
Castor oil, USP	7348	7324	7312

TABLE 5-continued

Batch Formulae for 0.15%, 0.45% and 0.6% testosterone gel formulations of the invention at the 8 kg Batch Size			
Components	Quantity per Batch (g)		
	0.15%	0.45%	0.6%
Oleoyl polyoxylglycerides, Ph. Eur./NF	320	320	320
Colloidal silicon dioxide, NF	320	320	320

Example 6

Manufacturing Process and Process Controls

Testosterone Gel Formulations of the Invention

[0266] Material is manufactured according to the process shown in FIG. 40.

Mixing of the Ingredients—Bulk Gel

[0267] The Pre-Mix is prepared by mixing, with a propeller mixer, the full amount of Testosterone with portion 1 of the castor oil for 10 minutes.

[0268] Mixture I is prepared by adding the Pre-Mix to the remaining castor oil and mixing for 60 minutes. The product temperature is maintained below 50° C. for the entire mixing process.

[0269] The oleoyl polyoxylglycerides are pre-heated to 40-50° C. and mixed for 10 minutes before being added to Mixture I. This is identified as Mixture II. It is mixed for 45 minutes while maintaining product temperature below 50° C. Mixture II is then screened through a sieve to remove any un-dissolved Testosterone aggregates.

[0270] Mixture III is prepared by adding the colloidal silicon dioxide to Mixture II and mixing for 15 minutes while maintaining product temperature below 50° C. A visual check is conducted after this step, to ensure that the gel is clear.

[0271] At the completion of mixing the gel is stirred and cooled to a product temperature below 30° C. The product is then discharged into stainless steel drums and the bulk gel sample is taken for analytical testing.

Filling and Packaging—Clinical Supplies

[0272] After release of the final gel mixture by the quality control laboratory, the filling and packaging process is carried out by filling a pre-determined volume into the syringe followed by the application of the syringe cap. Two syringes are packaged into a foil pouch.

[0273] The syringes are filled using a pipette with the gel taken from a holding tank. The tip of the pipette is discarded after the syringe is filled and the syringe cap is applied. Each syringe is individually labelled.

[0274] Following the application of the label, two syringes are packaged in a pre-formed foil pouch and the pouch is sealed. Each pouch is labeled.

Example 7

Evaluation of Testosterone Gel Formulations of the Invention in Women with Anorgasmia

[0275] The pharmacokinetics and pharmacodynamic efficacy of testosterone gel formulations of the invention are

evaluated in studies of women with anorgasmia. The effect of testosterone gel formulations of the invention on sexual stimuli in women with anorgasmia is also determined.

[0276] Patient Population

[0277] Otherwise healthy females, aged 18 to 65 years, presenting with anorgasmia are evaluated. Sixteen (16) subjects are recruited.

[0278] Dosing

[0279] Three doses of testosterone gel formulations of the invention are investigated: 150 µg, 450 µg and 600 µg per nostril. A total of 5 doses of a testosterone gel formulation of the invention is administered BID intranasally to women. Placebo testosterone gel formulation of the invention is administered as a control.

[0280] Treatment Duration

[0281] Test subjects receive 5 doses of a testosterone gel formulation of the invention over a three day period.

[0282] Endpoints

[0283] Primary End-Point:

[0284] The plasma concentrations of total testosterone and dihydrotestosterone are measured using validated LC/MS/MS. The following pharmacokinetic parameters are determined for all subjects:

[0285] C_{min}, C_{max}, t_{max}, PTF and PTS are determined, for each dosing interval

[0286] AUC_{0-τ}, and C_{avg}, are calculated for each dosing interval.

[0287] The percentage of time within, below, and above the physiological reference range for plasma testosterone and dihydrotestosterone.

[0288] Secondary End-Points:

[0289] Efficacy is determined by a battery of computer and psychophysiological tests.

[0290] Safety is monitored according to the following parameters:

[0291] Complete blood counts at Baseline and the Close-Out Visit.

[0292] Clinical chemistry and urinalysis testing at Baseline and Close-Out assesses selected endocrine parameters, renal function, liver function, skeletal/heart muscle damage, lipid abnormalities, and changes in calcium homeostasis.

[0293] Measurement of plasma testosterone, dihydrotestosterone and various hormones at Baseline, study days and Close-Out.

[0294] Adverse Events.

[0295] Randomization

[0296] Subjects in the ANOR cohort are randomized to receive either a testosterone gel formulation of the invention (3 dose levels) or placebo. Randomization is according to the design allocation below.

ANOR Cohort (Pre- and Post-menopausal)	Testosterone Gel Formulation of the invention	Testosterone Gel Formulation of the invention	Testosterone Gel Formulation of the invention	Placebo BID
	LD	MD	HD	
	BID	BID	BID	

LD (0.15%)—low dose;

MD (0.45%)—mid dose;

HD (0.6%)—high dose

[0297] Blinding

[0298] The analysis is both a double-blind and open label study, depending on the treatment cohort. For subjects in the ANOR group, the study is placebo-controlled and double-blinded.

[0299] Dosage and Dosage Regimen

[0300] All subjects are administered a testosterone gel formulation of the invention (0.15%, 0.45% or 0.6%) or testosterone gel formulation of the invention placebo on five (5) occasions during the study: Day 1 at 2000 hours, Day 2 at 800 and 2000 hours, and Day 3 at 800 and 2000 hours. The intranasal gel is administered to both nostrils (1 syringe (100 µl volume) per nostril).

[0301] Package and Labelling

[0302] Study medication consists of a testosterone gel formulation of the invention and placebo gel and is packed in single use syringes designed to expel 100 µl of gel. Two syringes are packaged in a foil pouch.

[0303] Treatment Schedule

[0304] Subjects are randomized into the dosing regimen that is administered during a four day (three night) in-patient treatment period and receive either an intranasal testosterone gel formulation of the invention (3 dose levels) or placebo (ANOR) according to the design allocation:

ANOR Cohort	Testosterone Gel Formulation of the invention	Testosterone Gel Formulation of the invention	Testosterone Gel Formulation of the invention	Placebo BID
	LD	MD	HD	
	BID	BID	BID	

[0305] The randomization scheme is created for each study center and will consist of blocks of four treatments per cohort.

[0306] The study is a four day study. The study starts with Study Drug dosing between 2000 and 2100 hours on Day 1 (Baseline). Blood samples for plasma testosterone and dihydrotestosterone profiles are drawn at -60, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 minutes following the evening dose on Days 1 and 3 and at 0 and 60 minutes post administration following the morning/evening doses on Day 2 and morning dose on Day 3.

[0307] For subjects allocated to the three testosterone gel formulation of the invention arms or the placebo gel, PD testing takes place on Day 2, 30 minutes and 4.5 hours after the morning dose (psychophysiological testing) and Day 3, 30 minutes after the morning dose (computer testing). Subjects undergo a practice psychophysiological session before the first dosing.

[0308] Because subjects are expected to not sleep well during their first overnight stay with repeated nightly blood draws, the order of psychophysiological testing and computer testing is not counterbalanced. Computer testing is expected to be more negatively affected by sleep deprivation than psychophysiological testing. Therefore, on Day 2 psychophysiological testing takes place, and computer testing occurs on Day 3.

[0309] Adverse events are assessed and reported.

[0310] Patient Selection and Withdrawal

[0311] The subjects in this study are women with ANOR. Subjects are recruited from the medical practice or the general population through advertisements in local newspapers with

additional information available on a website. Before scheduling the screening visit, subjects are asked a series of standardized questions by telephone to assess whether they are likely to be suitable for the study.

[0312] Inclusion Criteria

[0313] Women aged 18-65 years.

[0314] Diagnosis of Female Orgasmic Disorder (Anorgasmia) according to the DSM-IV criteria. The current episode must be at least 24 weeks in duration by the Screening Visit. Subtype should be generalized and not due to etiological factors that would prohibit treatment response (e.g., depression, alcoholism, surgery, injury). HSDD as a co-morbid disorder is allowed only if it began after the anorgasmia.

[0315] BMI ≤ 35 .

[0316] Women must have a score of >11 on the FSDS-R at the Screen Visit together with a score of <26.55 on the FSFI.

[0317] Women in a steady relationship of at least 12 months or single women who use masturbation as the primary way to attempt to reach orgasm.

[0318] Pre- and post-menopausal women—for physiological and surgical post-menopausal women—estrogen/progestagen substitution (low dose combined ET/P) for at least three (3) months before study entry or post-menopausal women naïve to ET/P substitution.

[0319] Premenopausal heterosexual women will need to be on a reliable birth control method (i.e., OCPs or partner must use condoms).

[0320] Normal thyroid function, physiological prolactin concentration.

[0321] Normal otorhinolaryngologic examination.

[0322] Provide written informed consent.

[0323] Exclusion Criteria

[0324] History of any other clinically relevant psychiatric disorders that could impact sexual function, risks patient's safety, or may impact compliance are as assessed. This includes bipolar disorders, psychotic disorders, severe anxiety, eating disorders, antisocial personality disorders, etc.

[0325] History of Major Depressive Disorder within six (6) months prior the Screening Visit or a score of ≥ 14 on the Beck Depression Inventory II.

[0326] Subjects who meet DSM-IV criteria (APA) for Sexual Aversion Disorder, Substance-Induced Sexual Dysfunction, Dyspareunia (not caused by inadequate foreplay stimulation or alleviated by lubricants), Vaginismus, Gender Identity Disorder, Paraphilia, or for Sexual Dysfunction Due to a General Medical Condition.

[0327] Subjects experiencing relational discord as indicated by a score of ≥ 20 on the MMQ.

[0328] Subjects with known active pelvic inflammatory disease, urinary tract or vaginal infection/vaginitis, cervicitis, interstitial cystitis, vulvodynia, or significant vaginal atrophy.

[0329] Subjects who are breast feeding or have breast fed within the last six (6) months prior to the Baseline Visit.

[0330] Subjects who are pregnant (by serum pregnancy test at the Screen Visit) or have been pregnant within the last 12 months prior to the Baseline Visit.

[0331] Treatment with systemic glucocorticoids.

[0332] Treatment with sex steroid hormones such as androgens, estrogens other than in low dose combined

ET/P, or gestagens (e.g. anabolic steroids, DHEA, Premarin® (conjugated equine estrogens)).

[0333] Treatment with thyroid hormones (only for stable replacement therapy).

[0334] Significant intercurrent disease of any type, in particular liver, kidney, or heart disease, any form of diabetes mellitus (subjects using antacids or with treated hyperlipidaemia or treated hypothyroidism will not be excluded provided they have been stable on their drug dose for at least six (6) months).

[0335] History of nasal disorders (e.g., seasonal or perennial allergic rhinitis, atrophic rhinitis, polyposis, abuse of nasal decongestants, clinically relevant nasal septum deviation, recurrent epistaxis) or sleep apnea.

[0336] Subjects with a history of dementia or other neurodegenerative diseases, organic brain disease, stroke, transient ischemic attacks, brain surgery, significant brain trauma, multiple sclerosis, spinal cord injury, peripheral neuropathy, and epilepsy (febrile seizures limited to childhood do not exclude subjects).

[0337] History of cancer, excluding basal cell carcinoma.

[0338] History of severe or multiple allergies, severe adverse drug reaction or leucopenia.

[0339] History of abnormal bleeding tendencies or thrombophlebitis unrelated to venepuncture or intravenous cannulation.

[0340] History of DVT.

[0341] History of Hepatitis B, a positive test for Hepatitis B surface antigen, a history of Hepatitis C, a positive test for Hepatitis C antibody, a history of HIV infection or demonstration of HIV antibodies.

[0342] Recent history of significant sleeping problems. Shift-worker need to have adequate day-night rhythms for three weeks before study entry.

[0343] Regular drinkers of more than three (3) units of alcohol daily (1 unit=300 ml beer, 1 glass wine, 1 measure spirit).

[0344] History of, or current evidence of, abuse of alcohol or any drug substance, licit or illicit; or positive urine drug and alcohol screen for drugs of abuse and alcohol.

[0345] Difficulty in abstaining from OTC medication (except occasional paracetamol/aspirin) for the duration of the study.

[0346] Poor compliers or subjects unlikely to attend study visits.

[0347] Receipt of any drug as part of a research study within 30 days of initial dose administration in this study.

[0348] Blood donation (usually 550 ml) within the 12 week period before the initial study dose.

[0349] Treatment of Subjects

[0350] Study Visits

[0351] Visit 1 (Day—15)—Screening Subjects for Inclusion and Exclusion Criteria:

[0352] Pre-study screening is carried out within two (2) weeks prior to the start of the treatment. Subjects, after having voluntarily signed the Informed Consent Form, and before enrolment, are interviewed by the Clinical Investigator or his/her designee physician who will take the medical, sexual and physical history, record demographic data, and perform a routine physical examination including vital signs (blood pressure, resting heart rate, body weight, and height).

- [0353] FSFI and FSDS-R are administered, as well as MMQ, BDI-II, ISS, SIDI-II, SESII-W.
- [0354] The otolaryngologic examination is done by an ENT specialist.
- [0355] Venous blood is collected, after an overnight fast, for a CBC (hemoglobin, hemoglobin A1c, hematocrit, MCV, MCHC, RBC, WBC & differential), Clinical Chemistry profile (Na/K, glucose, urea, creatinine, total bilirubin, albumin, calcium, phosphate, uric acid, LDL, HDL, triglycerides, AST, ALT, ALP, GGT and CK).
- [0356] Venous blood samples for estradiol, free testosterone, free testosterone (percent), follicle stimulating hormone, luteinizing hormone, prolactin, progesterone, sex hormone binding globulin, total testosterone, and dehydroepiandrosterone sulfate are collected.
- [0357] A blood sample for TSH, total and free tri-iodothyronine, total and free thyroxine is obtained.
- [0358] Urine is collected for measuring specific gravity, glucose, ketones, bilirubin, pH, urobilinogen, leukocytes, nitrites.
- [0359] Subjects undergo Hepatitis B, C and HIV testing (Hepatitis B surface antigen, Hepatitis C antibody, HIV antibodies in plasma).
- [0360] A urine drug screen is performed for amphetamines, benzodiazepines, cannabinoids, cocaine, opiates, MDMA. Subjects with positive test are not enrolled.
- [0361] Ethanol will be screened for by breathalyzer.
- [0362] Visit 2 (Day 1)—Start of Baseline, Randomization, PK Blood Sampling and PD Testing:
- [0363] Subjects are admitted to the clinic in the afternoon for three overnights stays.
- [0364] A check-in examination is conducted to check for disallowed medications
- [0365] (OTC and prescription), drugs, alcohol or cigarettes. Subjects are requested to abstain from alcohol for 48 hours prior to admission to the clinic. Alcohol consumption is strictly forbidden at any time during the overnight stay in the clinic. There are no restrictions with respect to food intake during the blood collections for the PK profile.
- [0366] Urine tests for the same drugs of abuse as at Screening are repeated.
- [0367] Pregnancy is excluded using a urine test (if applicable).
- [0368] Vital signs (blood pressure, resting heart rate, and body weight) are checked.
- [0369] Blood is drawn for a CBC, chemistry profile, hormone profile, and pregnancy testing.
- [0370] Urine for urinalysis and urine drug screen is collected along with performing an alcohol breath test.
- [0371] Before dosing and blood sampling, subjects undergo a psychophysiological familiarization test in which neutral and erotic films are shown and VPA is recorded to get acquainted with the experimental procedures and being exposed to explicit erotic stimuli. The data obtained is not be used for analysis.
- [0372] A venous cannula will be placed in a forearm vein, and blood sampling starts one hour before the evening administration of the Study Drug.
- [0373] Subjects are dosed between 2000 and 2100 hours.
- [0374] Blood samples are drawn for plasma testosterone and dihydrotestosterone levels at -60, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes post administration.
- [0375] Randomization to the treatment schemes is done at this visit.
- [0376] Safety assessment is recorded.
- [0377] Subjects remain in the clinic overnight.
- [0378] Visit 3 (Day 2)—PK Blood Sampling:
- [0379] Vital signs are obtained.
- [0380] A hormone profile is collected.
- [0381] Subjects are dosed with Study Drug between 800 and 900 and between 2000 and 2100 hours (unless on Intrinsa®). Blood samples are drawn for plasma testosterone and dihydrotestosterone levels at times 0 and 60 minutes post administration.
- [0382] For subjects allocated to the three testosterone gel formulation of the invention—arms or the placebo gel, psychophysiological testing is performed 30 minutes and 4.5 hours post-dosing in the morning
- [0383] Safety assessment is recorded.
- [0384] Subjects remain in the clinic overnight.
- [0385] Visit 4 (Day 3)—PK Blood Sampling and PD Testing:
- [0386] Vital signs are obtained.
- [0387] A hormone profile is collected.
- [0388] Subjects are dosed with Study Drug at 800 and 900 and between 2000 and 2100 hours. Blood samples are drawn for plasma testosterone and dihydrotestosterone levels at times 0 and 60 minutes following administration of the morning dose and at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes following administration of the evening dose.
- [0389] For subjects allocated to the three testosterone gel formulation of the invention—arms or the placebo gel, computer testing is performed 30 minutes post-dosing in the morning.
- [0390] Safety assessment is recorded.
- [0391] Visit 5 (Day 4)—Discharge-Close Out:
- [0392] Physical examination including vital signs.
- [0393] Venous blood is collected, after an overnight fast, for a CBC (hemoglobin, hemoglobin A1c, hematocrit, MCV, MCHC, RBC, WBC & differential), clinical chemistry profile (Na/K, glucose, urea, creatinine, total bilirubin, albumin, calcium, phosphate, uric acid, LDL, HDL, triglycerides, AST, ALT, ALP, GGT and CK).
- [0394] Appropriate blood samples for estradiol, free testosterone, free testosterone (percent), follicle stimulating hormone, luteinizing hormone, prolactin, progesterone, sex hormone binding globulin, total testosterone, and dehydroepiandrosterone sulfate are collected.
- [0395] Urine is collected for measuring specific gravity, glucose, ketones, bilirubin, pH, urobilinogen, leukocytes, nitrites.
- [0396] Safety assessment is recorded.
- [0397] Clinical Assessments
- [0398] Screening and Covariate Questionnaires are used for clinical assessments.
- BDI
- [0399] To index the current level of depressive symptoms, the 21-item BDI-II is administered (Beck, Steer, & Brown.

1996), Dutch adaptation (Van der Does, 2002). The range for the BDI total score is 0-63, with higher scores indicating more depressive symptoms.

MMQ

[0400] The Maudsley Marital Questionnaire (MMQ; Crowe, 1978) is a 20-item self-report instrument measuring dissatisfaction with the general relationship, with the sexual relationship, and with life in general. The MMQ has shown good reliability and validity. The psychometric qualities of the Dutch version of the MMQ were also found to be satisfactory (Arrindell, Boelens, & Lambert, 1983). Higher scores represent larger dissatisfaction.

FSFI

[0401] The level of the woman's sexual functioning is assessed by the Female Sexual Function Index (FSFI; Rosen, Brown, Heiman, et al. 2000). The FSFI© is a self-administered questionnaire that consists of 19 questions. The scale contains six domains: desire, arousal, lubrication, orgasm, satisfaction, and pain. The range for the total score is 2-36, with lower scores representing worse sexual function. The psychometric quality of the FSFI is satisfactory (Wiegel, Meston, & Rosen, 2005). Based on a Dutch sample consisting of approximately 350 women with and without sexual complaints, the internal consistency and stability of the FSFI were found to be satisfactory-to-good. The FSFI's ability to discriminate between sexually functional and dysfunctional women was excellent as was the ability to predict the presence or absence of sexual complaints (ter Kuile, Brauer, & Laan, 2006).

FSDS-R

[0402] The woman's level of personal distress due to sexual dysfunction is assessed by the Female Sexual Distress Scale-Revised (FSDS-R©; Derogatis, Clayton, Lewis-D'Agostino, et al. 2008).

[0403] The items inquire about negative feelings and problems that are bothersome or cause distress during the past 30 days. Reliability and validity of the FSDS© (12-item version) has been evaluated in different samples of sexually functional and dysfunctional women. For the FSDS©, results indicated a unidimensional factor structure, a high degree of internal consistency, and test-retest reliability. The FSDS© showed a high degree of discrimination between sexually dysfunctional and functional women in each of its three validation studies. Results in a Dutch sample supported the unidimensional structure of the FSDS and its reliability and psychometric validity (ter Kuile, Brauer, & Laan, 2006). An additional question (question 13) has been added to the validated FSDS©. This question is about distress specifically related to sexual desire. The maximum total score of the FSDS-R© indicating the maximum level of sexual distress is '52'. Both the FSDS-R© total score and the Question 13 score alone will be analyzed.

[0404] Covariate Questionnaires

Index of Sexual Satisfaction (ISS)

[0405] The woman's level of sexual satisfaction as assessed by the Index of Sexual Satisfaction (ISS; Hudson, Harrison, & Crosscup, 1981). This 25-item questionnaire asks subjects to evaluate various aspects of their sexual relationship, leading to a sum score that can range between 0 and 100. Higher

scores correspond to greater sexual satisfaction. This measure has been shown to have good face, convergent, and discriminant validity with various samples. Example items are "I feel that my partner enjoys our sex life," "I think that sex is wonderful," and "My partner is sexually very exciting." For the purpose of the present study, the ISS is translated into Dutch.

SDI-II

[0406] The level of the woman's sexual desire is assessed by the Sexual Desire Inventory-II (SDI-II; Spector, Carey, & Steinberg, 1996). The SDI-II consists of two seven-item self-report scales: the Dyadic Sexual Desire scale, which measures an individual's desire for sexual activity with a partner, and the Solitary Sexual Desire scale, which measures an individual's desire for autoerotic sexual activity. The two subscales were internally consistent (Cronbach's α : Dyadic scale=0.86; Solitary scale=0.96).

Sexual Excitation/Sexual Inhibition (SESII-W)

[0407] The Sexual Inhibition/Excitation Inventory for women (SESII-W; Graham, Sanders, & Milhausen, 2006) is used to assess individuals' propensity for sexual excitation and sexual inhibition. It consists of 36 items, referring to stimulus situations that could affect sexual inhibition and sexual excitation or to general statements about arousability and inhibition. The instructions ask women to report what would be the most typical reaction now or how they think they would respond if the item does not apply to them. Items are rated on a 4-point Likert-rating scale, from "strongly disagree" to "strongly agree." The SESII-W has eight lower-order factors, which in turn load on two higher-order factors, Sexual Excitation and Sexual Inhibition. The questionnaire shows good test-retest reliability and convergent and discriminant validity and Sexual Excitation and Sexual Inhibition appear to be relatively independent factors. The list is already in use in the Netherlands, but psychometric properties have not been investigated yet.

[0408] Efficacy Pharmacodynamic Testing

Computer Testing

Single Target Implicit Association Task (StIAT):

[0409] Conform Wigboldus et al. (2005), the stIAT used in this study is designed to assess subjects' affective associations with sexual stimuli (Brauer, van Leeuwen, Janssen, et al. submitted). Subjects are instructed to classify pictures portraying sexual acts (i.e., target stimuli) and words representing "positive" or "negative" meanings (i.e., attribute stimuli) to the appropriate superordinate category (i.e., "sex", "positive", "negative") as quickly as possible by pressing only a left or right response key on a keyboard. These labels used for these categories (sex, positive, negative) are continuously visible on the computer screen. The stIAT consists of a combination of practice and experimental blocks (see Greenwald, McGhee & Schwartz, 1998 for detailed methodology). The experimental blocks consist of one 'incongruent' and one 'congruent' block of trials. In the incongruent block, "sex" and "negative" are mapped on a single key and "positive" on the other, while in the congruent block, "sex" and "positive" are mapped on the same key and "negative" on the other. The difference in reaction times between the two experimental blocks is assumed to reflect whether sex is associated more

strongly with either positive or negative. Faster responses in the congruent block (compared to the other block) reflect stronger associations between positive and sex, and faster responses in the incongruent block reflect stronger associations between negative and sex. The target-attribute combinations that share response keys (i.e., block order), and left or right key response requirements are counterbalanced. Each critical block consists of 40 trials of which responses were divided equally over the two response keys. The target category consists of 5 exemplar stimuli of sexual images from the International Affective Picture System (IAPS; Center for the Study of Emotion and Attention, 1995), with the following numbers: 4800, 4652, 4658, 4659, and 4672. The attribute categories consists of 20 generally positive and 20 generally negative words (Dotsch & Wigboldus, 2008; Dotsch, Wigboldus, Langner et al. 2008), thus reflecting more global affective associations with sex. These words were controlled for length and frequency. With respect to the validity, the stLAT's strength lies in high effect sizes due to double opposing categories often leading to slower reaction times (the categorization decision requires effort as there are several possibilities to consider).

Picture Association Task (PAT)

[0410] This task, developed by van Leeuwen and Macrae (2004), is based on the Affective Priming Task (e.g., Bargh, Chaiken, Govender, et al. 1992; Fazio, Sanbonmatsu, Powell, et al. 1986; Hermans, De Houwer, & Eelen, 1994) where target words are preceded by another word or image that influences the categorisation speed of the target word. In the PAT, however, target words and images appear simultaneously. In the PAT used in this study, subjects are presented with positive or negative words superimposed on either sexual or neutral pictures (Brauer, van Leeuwen, Janssen, et al. submitted). They are instructed to categorize the words as fast as possible as either positive or negative by pressing one of two computer keys. Subjects are further instructed to focus on the words that appeared on the screen and not to attend to the background images as these are of no importance for the task and the categories to which the pictorial stimuli belong (sex, neutral) are not explained. Thus, the PAT captures the unintentional influence of the affective value of the pictorial background stimuli on task performance. The time to select the correct response to the words (positive or negative) is influenced by the match between the valence of the word and the valence of the background image (sex or neutral), thereby revealing indirectly the valence of the picture for the subjects. The word categories consist of 10 positive words and 10 negative words. Whereas for the stLAT general positive and negative words are selected (e.g., peace, respect, war, hate), the PAT consists of positive and negative words that are applicable to a sexual situation, but that do not exclusively refer to sexual experiences (e.g., enjoyable, wonderful, dirty, disgusting) in order to create a conceptual overlap between the content of the words and the content triggered by the sexual pictures. These words are taken from a pilot study in the Netherlands in which female subjects (N=20) were asked to indicate on a 7-point Likert scale for each positive and negative word how well it described a positive or a negative sexual situation, respectively (Brauer & Laan, 2008). The words appear at one of four randomized locations on the picture to avoid expectation-related responses and to make sure subjects would move their eyes over the image. The sexual pictures were taken from another study on implicit

associations with sexual stimuli in women with dyspareunia (Brauer, de Jong, Huijding et al., 2009). These pictures display a variety of sexual acts (e.g., kissing, cunnilingus, fellatio, coitus). Based on each sexual picture, a control picture was created by scrambling the sexual image, leaving a neutral stimulus. All pictures are standardized to 600x480 pixels and matched for brightness and contrast. Each stimulus remains on the screen until subjects make a decision or until 3,000 ms has elapsed. After 10 practice trials, 80 experimental trials are presented. Each word is paired randomly with a sexual picture and a neutral picture, resulting in four different combinations each presented 20 times: positive words and sexual images, negative words and sexual images, positive words and neutral images, negative words and neutral images. The order of presentation of the trials is counterbalanced within, and response key mappings (i.e., positive/negative or negative/positive) are counterbalanced across subjects. The computer records the accuracy and latency of each response. With respect to validity, the strength of the PAT is that it is not sensitive to a possible interpretation bias due to the need to attend to the different stimulus categories at the same time, as is the case in the stLAT.

Dot Probe Task (DOT)

[0411] The dot-probe task (DOT) assesses attentional preference for sexual and neutral visual stimuli. In this task, subjects are shown two images side by side on a computer screen for 500 ms. When the two images disappear, a target stimulus represented by a small dot appears in the place of one of the images. Subjects are asked to indicate the location (side) of the dot. Mean RTs are calculated for three categories: 1) neutral neutral 2) neutral sex with the dot under neutral 3) neutral sex with the dot under sex. If reaction times are faster when the dot appears in the place of a certain class of stimuli this indicates an attentional bias towards this class of stimuli.

Psychophysiological Testing

Genital Response (VPA)

[0412] Psychophysiological testing consists of assessment of genital response (vaginal pulse amplitude) and subjective sexual arousal during sexual to self-induced erotic fantasy (3 min), a low-intensity erotic film clip (5 min), and a high-intensity erotic film clip (5 min) (Laan et al., in preparation). The erotic conditions are separated by variable interstimulus intervals during which subjects complete a concentration task (simple arithmetic problems) to allow for return-to-baseline. The erotic stimulus testing is preceded by a 8 min neutral film to establish baseline levels. VPA is measured using a vaginal photoplethysmograph developed by Bert Molenkamp (Technical Support, Department of Psychology, University of Amsterdam) based on instruments initially developed by Sincchak and Geer (1975). The light source (3 mm LED, $\lambda=620$ nm) and optical sensor (Texas Instruments TSL250) are produced in batches of 100, resulting in all photoplethysmographs used in this study having nearly equal electronic characteristics. A signal-conditioning amplifier separates the VPA from the direct current component using a 12 dB/octave, 0.7-Hz filter. Additional filtering for VPA is 24 dB/octave, 0.4 Hz high-pass. The VPA signal is digitalized at 100 Hz with a Keithley KPCI3107 A/D converter, running on a Windows 2000 PC system. Depth of the probe and orientation of the light source is controlled by a device (a 9-x2-cm FDA-ap-

proved perspex plate) attached to the cable within 5 cm of the optical sensor. Subjects are instructed to insert the probe until the plate touched their labia. The probe and plate are sterilized according to standard department protocol.

Sexual Feelings and Affect (SAQ).

[0413] Prior to and immediately after erotic stimulus subjects fill out a questionnaire measuring sexual feelings and affect during sexual stimulation, consisting of 5 scales: sexual arousal (Cronbach's $\alpha=0.87$); genital sensations (Cronbach's $\alpha=0.96$); sensuality (Cronbach's $\alpha=0.73$); positive affect (Cronbach's $\alpha=0.93$); and negative affect (Cronbach's $\alpha=0.65$). Each question is preceded by the sentence: "During the film, I felt:" after which a positive, negative, physical or sexual experience is described, for instance, pleasant; worried; genital pulsing or throbbing; sexually aroused. The items are measured on a 1 (not at all) to 7 (intensely) scale.

Acute Female Sexual Desire (AFSDQ)

[0414] Prior to and following psychophysiological testing subjects fill out the Acute Female Sexual Desire Questionnaire (Laan, Heiman, unpublished). This questionnaire assesses sexual interest in erotic stimuli and has shown to discriminate between women with acquired HSDD and sexually functional controls (Laan et al., in preparation)

[0415] Statistical Methodology

[0416] Calculation of Pharmacokinetic Parameters

[0417] C_{min} , C_{max} , and t_{max} are taken from the actual measured values. Values are determined relative to the testosterone administration time in treated subjects.

[0418] Area under the concentration curve (AUC) are estimated for the 0 to 24 hour time interval, as well as the BID dosing intervals, using the trapezoidal rule.

[0419] PK evaluations after Day 1 evening dose for testosterone gel formulation of the invention and placebo and Day 3 evening dose for testosterone gel formulation of the invention, placebo and Intrinsa® patch (which was applied on Day 1)—AUC, concentrations of total and free testosterone, DHT, estradiol, SHBG. Analyses of C_{avg} , C_{min} , C_{max} , t_{max} , $AUC_{0-\tau}$, PTF, and PTS. C_{avg} are calculated for the 12 hour period as well as τ when appropriate. For subjects on Intrinsa®, a 24 hour calculation is performed.

[0420] The average concentration in the dosing interval (C_{avg}) is calculated from the AUC using the following formula: $C_{avg} = AUC_{0-\tau} / \tau$, with τ =dosing interval time.

[0421] Peak Trough Fluctuation (PTF) and Peak Trough Swing (PTS) are calculated as follows:

$$PTF = (C_{max} - C_{min}) / C_{avg}$$

$$PTS = (C_{max} - C_{min}) / C_{min}$$

[0422] Percent time that the plasma testosterone concentration is above, within, and below the reference range of 10 to 70 ng/dl, is calculated.

[0423] Statistical Analysis of Pharmacodynamic Data stIAT:

[0424] Incorrect responses are excluded from analyses. In addition, RTs shorter than 300 ms or longer than 3000 ms are excluded from analyses. With respect to the stIAT data, Wigboldus, Holland & van Knippenberg (2005) is followed in that, for each subject, the median response latency of the correct responses to the attribute items in congruent and incongruent blocks is used. Following this, median reaction

times of the two experimental blocks are subtracted from one another to obtain a stIAT effect (i.e., stIAT effect=median (Sex/Negative)–median (Sex/Positive)). Negative stIAT effects indicate relatively stronger negative associations with sexual stimuli.

[0425] The stIAT effect is analyzed with an analysis of variance with fixed factor treatment, group (HSDD and SA) and the interaction treatment by group. The contrasts are calculated within the model.

PAT:

[0426] Median response latencies of the correct responses are calculated, following van Leeuwen and Macrae (2004). To correct for baseline reactions to positive and negative words, difference scores are calculated by subtracting RTs for neutral words superimposed on sexual pictures from positive words superimposed on sexual pictures. The same is done for negative words superimposed on sexual and neutral pictures (i.e., Sex/+RT (sex/positive words)–RT (neutral/positive words) and Sex/–RT (sex/negative words)–RT (neutral/negative words). Sex/+<Sex/–=automatic positive associations with sex).

[0427] The two PAT variables (RT positive and RT negative) are analyzed with an analysis of variance with fixed factors treatment, group (ANOR) and group by treatment. The contrasts will be calculated within the model.

DOT:

[0428] For each subject the difference between mean RT for the category neutral sex with the dot under sex and the mean RT for the category neutral sex with the dot under neutral is calculated to obtain a DOT effect (i.e., DOT effect=mean neutral sex with dot under neutral–mean neutral sex with dot under sex). Higher DOT scores indicate relatively stronger attention for sexual stimuli.

[0429] The DOT effect is analyzed with an analysis of variance with fixed factor treatment, group (ANOR) and the interaction treatment by group. The contrasts are calculated within the model.

VPA:

[0430] After VPA artefact deletion, done by a computer program developed Bert Molenkamp (Technical Support, Department of Psychology, University of Amsterdam), peak-to-trough amplitude is calculated for each remaining pulse. VPA is averaged every 30 seconds during several conditions: neutral film (8 min), self induced erotic fantasy (3 min), low intensity erotic film clip (5 min) and high intensity erotic film clip (5 min). All conditions are offered twice: once 0.5 hours after application of the nasal gel and once 4.5 hours after application of the nasal gel.

[0431] VPA during the erotic fantasy, the low intensity film and the high intensity film are analyzed separately and the different moments (0.5 hours after and 4.5 hours after dosing) are also be analyzed separately, resulting in 6 analyses. VPA during a condition and a moment is analyzed with a mixed model analysis of variance with fixed factors treatment, group (ANOR), time, group by treatment, treatment by time and random factor subject and the average VPA score during the neutral film as covariate. Contrasts are calculated within the model.

SAQ:

[0432] For each of the five SAQ scale mean response during a condition and a moment are analyzed with an analysis of variance with factors treatment, group (ANOR) and group by treatment, with the score prior to sexual stimuli as covariate. Contrasts are calculated within the model.

AFSDQ:

[0433] ASFDQ score after erotic stimulation during a moment is analyzed with an analysis of variance with factors treatment, group (ANOR), and group by treatment; and the score prior to sexual stimuli as covariate. Contrasts are calculated within the model.

[0434] If necessary to meet requirements for analysis of variance data is log-transformed. Results are back-transformed and reported as % change.

[0435] Graphs of least square means estimates over time by treatment are presented with error bars indicating the upper and lower 95% confidence interval for the highest and lowest profile respectively. Least square means of the contrasts are tabulated.

[0436] If analyses are not feasible according to the described models with the given data, analyses are adjusted. If considered useful extra exploratory analyses are conducted.

[0437] Statistical Analysis of Safety Data

[0438] Nasal Tolerance: Nasal tolerance data is presented in summary tables. No statistical analysis will be performed.

[0439] Vital Signs and Clinical Laboratory Parameters:

[0440] A table summarizing all laboratory test values and changes from Baseline is presented for each treatment group. In case parameters are $\pm 20\%$ of their reference range, the clinical significance of these findings are evaluated.

[0441] The results of this analysis are presented in FIGS. 1, and 4-6. The following FIGS. 3 and 9-11 compare the results between the effects of the lower dosage strength testosterone gel nasal formulations of the present invention in subjects diagnosed with anorgasmia or HSDD.

Example 8

Evaluation of a Testosterone Nasal Gel Formulation of the Invention in Women with HSDD

[0442] The pharmacokinetics and pharmacodynamic efficacy of testosterone gel formulations of the invention are evaluated in studies of women with HSDD. The effect of testosterone gel formulations of the invention on sexual stimuli in women with HSDD is also determined.

[0443] Dosing

[0444] Three doses of a testosterone gel formulation of the invention are investigated: 150 μg , 450 μg and 600 μg per nostril. A total of 5 doses of a testosterone gel formulation of the invention is administered BID intranasally to women. The Intrinsa® patch (300 μg testosterone) is administered as a control in the HSDD cohort.

[0445] Patient Population

[0446] Otherwise healthy females, aged 18 to 65 years, presenting with HSDD are evaluated. Sixteen (16) subjects are recruited to each indication.

[0447] Treatment Duration

[0448] Test subjects receive 5 doses of a testosterone gel formulation of the invention over a three day period.

[0449] Endpoints

[0450] Primary End-Point:

[0451] The plasma concentrations of total testosterone and dihydrotestosterone are measured using validated LC/MS/MS. The following pharmacokinetic parameters are determined for all subjects:

[0452] C_{min}, C_{max}, t_{max}, PTF and PTS are determined, for each dosing interval

[0453] AUC_{0- τ} , and C_{avg}, are calculated for each dosing interval.

[0454] The percentage of time within, below, and above the physiological reference range for plasma testosterone and dihydrotestosterone.

[0455] Secondary End-Points:

[0456] Efficacy is determined by a battery of computer and psychophysiological tests.

[0457] Safety is monitored according to the following parameters:

[0458] Complete blood counts at Baseline and the Close-Out Visit.

[0459] Clinical chemistry and urinalysis testing at Baseline and Close-Out assesses selected endocrine parameters, renal function, liver function, skeletal/heart muscle damage, lipid abnormalities, and changes in calcium homeostasis.

[0460] Measurement of plasma testosterone, dihydrotestosterone and various hormones at Baseline, study days and Close-Out.

[0461] Adverse Events.

[0462] Randomization

[0463] Subjects in the HSDD cohort are randomized to receive either a testosterone gel formulation of the invention (3 dose levels) or the Intrinsa® patch. Randomization is according to the design allocation below.

HSDD Cohort (Post-menopausal)	Testosterone Gel Formulation of the invention LD BID	Testosterone Gel Formulations of the invention MD BID	Testosterone Gel Formulations of the invention HD BID	Intrinsa® Patch (one patch in total)
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LD (0.15%)—low dose;

MD (0.45%)—mid dose;

HD (0.6%)—high dose

[0464] Blinding

[0465] This is both a double-blind and open label study, depending on the treatment cohort. For the HSDD cohort, this is a partly open-label study, as blinding of intra-nasal dosing versus patch dosing is not feasible. The testosterone gel formulation of the invention dose in the HSDD cohort is blinded.

[0466] Dosage and Dosage Regimen

[0467] Three-quarters (75%) of the subjects in the HSDD cohort are administered a testosterone gel formulation of the invention (0.15%, 0.45% or 0.6%) on five (5) occasions during the study: Day 1 at 2000 hours, Day 2 at 800 and 2000 hours, and Day 3 at 800 and 2000 hours. The intranasal gel is administered to both nostrils (1 syringe (100 μl volume) per nostril). To the remaining one-quarter (25%) of the subjects is administered the Intrinsa® patch at 2000 hours on Day 1 which will remain on the subject's lower abdomen for the duration of the study. The patch is removed on Day 4 prior to discharging the subject from the clinic.

[0468] Package and Labelling

[0469] Study medication consists of testosterone gel formulations of the invention and testosterone gel formulations of the invention placebo gel and is packed in single use syringes designed to expel 100 µl of gel. Two syringes are packaged in a foil pouch. The active control for the HSDD cohort, Intrinsa®, remains in its original packaging from the manufacturer.

[0470] Treatment Schedule

[0471] Subjects are randomized into the dosing regimen that is administered during a four day (three night) in-patient treatment period and receive either intranasal testosterone gel formulation of the invention (3 dose levels) or Intrinsa® patch (HSDD) according to the design allocation:

HSDD Cohort	Testosterone Gel Formulations of the invention	Testosterone Gel Formulations of the invention MD	Testosterone Gel Formulations of the invention	Intrinsa® Patch (one patch in total)
	LD	BID	HD	
	BID		BID	

[0472] The randomization scheme is created for each study center and consists of blocks of four treatments per cohort.

[0473] The study is a four day study. The study start with Study Drug dosing between 2000 and 2100 hours on Day 1 (Baseline). Blood samples for plasma testosterone and dihydrotestosterone profiles are drawn at -60, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 minutes following the evening dose on Days 1 and 3 and at 0 and 60 minutes (except Intrinsa arm—no 60 minute on Day 2 and Day 3 morning draws) post administration following the morning/evening doses on Day 2 and morning dose on Day 3.

[0474] For subjects allocated to the three testosterone gel formulations of the invention arms or the placebo gel, PD testing will take place on Day 2, 30 minutes and 4.5 hours after the morning dose (psychophysiological testing) and Day 3, 30 minutes after the morning dose (computer testing). Subjects will have undergone a practice psychophysiological session before the first dosing.

[0475] Because subjects are expected to not sleep well during their first overnight stay with repeated nightly blood draws, the order of psychophysiological testing and computer testing is not counterbalanced. Computer testing is expected to be more negatively affected by sleep deprivation than psychophysiological testing. Therefore, on Day 2 psychophysiological testing takes place, and computer testing occurs on Day 3. For the subjects randomized to the Intrinsa® patch, psychophysiological testing take place on Day 3 between 800 and 900 hours and computer testing follows in the afternoon of Day 3 between 1600 and 1700 hours.

[0476] Adverse events are assessed and reported.

[0477] Patient Selection and Withdrawal

[0478] The subjects in this study are women with HSDD. Subjects are recruited from the medical practice or the general population through advertisements in local newspapers with additional information available on a website. Before scheduling the screening visit, subjects are asked a series of standardized questions by telephone to assess whether they are likely to be suitable for the study.

[0479] Inclusion Criteria

[0480] Women up to 65 years.

[0481] Postmenopausal women with the primary diagnosis of HSDD, generalized acquired type, according to DSM-IV criteria at the Screening Visit. The current episode must be at least 24 weeks in duration by the Screening Visit. Secondary Female Sexual Arousal Disorder and/or Female Orgasmic Disorder are allowed if comorbid. This inclusion criterion is met only if the HSDD began prior to Female Sexual Arousal Disorder and/or Female Orgasmic Disorder and the HSDD is of more importance to the subject, in the investigators' judgment.

[0482] BMI ≤ 35 .

[0483] Women must have a score of >11 on the FSDS-R at the Screen Visit together with a score of <26.55 on the FSFI.

[0484] Women in a steady relationship of at least 12 months

[0485] Physiological and surgical post-menopausal women—estrogen/progesterone substitution (low dose combined ET/P) for at least three (3) months before study entry or post-menopausal women naïve to ET/P substitution.

[0486] Normal thyroid function, physiological prolactin concentration.

[0487] Normal otorhinolaryngologic examination.

[0488] Provide written informed consent.

[0489] Exclusion Criteria—HSDD

[0490] History of any other clinically relevant psychiatric disorders that could impact sexual function, risk patient's safety, or may impact compliance, as assessed by the MINI. This includes bipolar disorders, psychotic disorders, severe anxiety, eating disorders, antisocial personality disorders, etc.

[0491] History of Major Depressive Disorder within six (6) months prior to Screening Visit or a score of ≥ 14 on the Beck Depression Inventory II.

[0492] Subjects who meet DSM-IV criteria (APA) for Sexual Aversion Disorder, Substance-Induced Sexual Dysfunction, Dyspareunia (not caused by inadequate foreplay stimulation or alleviated by lubricants), Vaginismus, Gender Identity Disorder, Paraphilia, or for Sexual Dysfunction Due to a General Medical Condition.

[0493] Subjects with known active pelvic inflammatory disease, urinary tract or vaginal infection/vaginitis, cervicitis, interstitial cystitis, vulvodynia, or significant vaginal atrophy.

[0494] Women with relationship discord as indicated by a score of ≥ 20 on the MMQ.

[0495] Treatment with systemic glucocorticoids.

[0496] Treatment with sex steroid hormones such as androgens, estrogens other than in low dose combined ET/P, or gestagens (e.g. anabolic steroids, DHEA, Premarin® (conjugated equine estrogens)).

[0497] Treatment with thyroid hormones (only for stable replacement therapy).

[0498] Significant intercurrent disease of any type, in particular liver, kidney, or heart disease, or any form of diabetes mellitus (subjects using antacids or with treated hyperlipidaemia or treated hypothyroidism will not be excluded provided they have been stable on their drug dose for at least six months).

- [0499] History of nasal disorders (e.g., seasonal or perennial allergic rhinitis, atrophic rhinitis, polyposis, abuse of nasal decongestants, clinically relevant nasal septum deviation, recurrent epistaxis) or sleep apnea.
- [0500] Subjects with a history of dementia or other neurodegenerative diseases, organic brain disease, stroke, transient ischemic attacks, brain surgery, significant brain trauma, multiple sclerosis, spinal cord injury, peripheral neuropathy, and epilepsy (febrile seizures limited to childhood do not exclude subjects)
- [0501] History of cancer, excluding basal cell carcinoma
- [0502] History of severe or multiple allergies, severe adverse drug reaction or leucopenia.
- [0503] History of abnormal bleeding tendencies or thrombophlebitis unrelated to venepuncture or intravenous cannulation.
- [0504] History of DVT.
- [0505] History of Hepatitis B, a positive test for Hepatitis B surface antigen, a history of Hepatitis C, a positive test for Hepatitis C antibody, a history of HIV infection or demonstration of HIV antibodies.
- [0506] Recent history of significant sleeping problems. shift-workers need to have adequate day-night rhythms for three weeks before study entry.
- [0507] Regular drinkers of more than three (3) units of alcohol daily (1 unit=300 ml beer, 1 glass wine, 1 measure spirit).
- [0508] History of, or current evidence of, abuse of alcohol or any drug substance, licit or illicit; or positive urine drug and alcohol screen for drugs of abuse and alcohol.
- [0509] Difficulty in abstaining from OTC medication (except occasional paracetamol/aspirin) for the duration of the study.
- [0510] Poor compliers or subjects unlikely to attend study visits.
- [0511] Receipt of any drug as part of a research study within 30 days of initial dose administration in this study.
- [0512] Blood donation (usually 550 ml) within the 12 week period before the initial study dose.

Treatment of Subjects

Study Visits

- [0513] Visit 1 (Day—15)—Screening Subjects for Inclusion and Exclusion Criteria:
 - [0514] Pre-study screening is carried out within two (2) weeks prior to the start of the treatment. Subjects, after having voluntarily signed the Informed Consent Form, and before enrolment, are interviewed by the Clinical Investigator or his/her designee physician who takes the medical, sexual and physical history, record demographic data, and performs a routine physical examination including vital signs (blood pressure, resting heart rate, body weight, and height).
 - [0515] FSFI and FSDS-R are administered, as well as MMQ, BDI-II, ISS, SIDI-II, SESII-W.
 - [0516] The otolaryngologic examination is done by an ENT specialist.
 - [0517] Venous blood is collected, after an overnight fast, for a CBC (hemoglobin, hemoglobin A1c, hematocrit, MCV, MCHC, RBC, WBC & differential), Clinical Chemistry profile (Na/K, glucose, urea, creatinine, total

- bilirubin, albumin, calcium, phosphate, uric acid, LDL, HDL, triglycerides, AST, ALT, ALP, GGT and CK).
- [0518] Venous blood samples for estradiol, free testosterone, free testosterone (percent), follicle stimulating hormone, luteinizing hormone, prolactin, progesterone, sex hormone binding globulin, total testosterone, and dehydroepiandrosterone sulfate are collected.
- [0519] A blood sample for TSH, total and free tri-iodothyronine, total and free thyroxine is obtained.
- [0520] Urine is collected for measuring specific gravity, glucose, ketones, bilirubin, pH, urobilinogen, leukocytes, nitrites.
- [0521] Subjects also undergo Hepatitis B, C and HIV testing (Hepatitis B surface antigen, Hepatitis C antibody, HIV antibodies in plasma).
- [0522] A urine drug screen is performed for amphetamines, benzodiazepines, cannabinoids, cocaine, opiates, MDMA. Subjects with positive test are not enrolled.
- [0523] Ethanol is screened for by breathalyzer.
- [0524] Visit 2 (Day 1)—Start of Baseline, Randomization, PK Blood Sampling and PD Testing:
 - [0525] Subjects are admitted to the clinic in the afternoon for three overnights stays.
 - [0526] A check-in examination is conducted to check for disallowed medications (OTC and prescription), drugs, alcohol or cigarettes. Subjects are requested to abstain from alcohol for 48 hours prior to admission to the clinic. Alcohol consumption is strictly forbidden at any time during the overnight stay in the clinic. There are no restrictions with respect to food intake during the blood collections for the PK profile.
 - [0527] Urine tests for the same drugs of abuse as at Screening are repeated.
 - [0528] Pregnancy is excluded using a urine test (if applicable).
 - [0529] Vital signs (blood pressure, resting heart rate, and body weight) are checked.
 - [0530] Blood is drawn for a CBC, chemistry profile, hormone profile, and pregnancy testing.
 - [0531] Urine for urinalysis and urine drug screen is collected along with performing an alcohol breath test.
 - [0532] Before dosing and blood sampling, subjects undergo a psychophysiological familiarization test in which neutral and erotic films are shown and VPA is recorded to get acquainted with the experimental procedures and being exposed to explicit erotic stimuli. The data obtained is not used for analysis.
 - [0533] A venous cannula is placed in a forearm vein, and blood sampling starts one hour before the evening administration of the Study Drug.
 - [0534] Subjects are dosed between 2000 and 2100 hours.
 - [0535] Blood samples are drawn for plasma testosterone and dihydrotestosterone levels at -60, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes post administration.
 - [0536] Randomization to the treatment schemes is done at this visit.
 - [0537] Safety assessment is recorded.
 - [0538] Subjects remain in the clinic overnight.

[0539] Visit 3 (Day 2)—PK Blood Sampling:

[0540] Vital signs are obtained.

[0541] A hormone profile is collected.

[0542] Subjects are dosed with Study Drug between 800 and 900 and between 2000 and 2100 hours (unless on Intrinsa®). Blood samples are drawn for plasma testosterone and dihydrotestosterone levels at times 0 and 60 minutes post administration. For subjects on Intrinsa®, a time 0 draw between 800-900 and 2000-2100 hours is obtained.

[0543] For subjects allocated to the three testosterone gel formulations of the invention—arms or the placebo gel, psychophysiological testing is performed 30 minutes and 4.5 hours post-dosing in the morning

[0544] Safety assessment is recorded.

[0545] Subjects remain in the clinic overnight.

[0546] Visit 4 (Day 3)—PK Blood Sampling and PD Testing:

[0547] Vital signs are obtained.

[0548] A hormone profile is collected.

[0549] Subjects are dosed with Study Drug at 800 and 900 and between 2000 and 2100 hours (unless on Intrinsa®). Blood samples are drawn for plasma testosterone and dihydrotestosterone levels at times 0 and 60 minutes following administration of the morning dose and at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes following administration of the evening dose. Subjects receiving Intrinsa® have the time 0 draw for the morning dose and all times in the evening as if they received a new dose.

[0550] For subjects allocated to the three testosterone gel formulations of the invention—arms or the placebo gel, computer testing is performed 30 minutes post-dosing in the morning. For the women randomized to the Intrinsa® patch, psychophysiological testing takes place on Day 3 between 800 and 900 hours. Computer testing follows in the afternoon of Day 3 between 1600 and 1700 hours.

[0551] Safety assessment is recorded.

[0552] Visit 5 (Day 4)—Discharge-Close Out:

[0553] Physical examination including vital signs.

[0554] Venous blood is collected, after an overnight fast, for a CBC (hemoglobin, hemoglobin A1c, hematocrit, MCV, MCHC, RBC, WBC & differential), clinical chemistry profile (Na/K, glucose, urea, creatinine, total bilirubin, albumin, calcium, phosphate, uric acid, LDL, HDL, triglycerides, AST, ALT, ALP, GGT and CK).

[0555] Appropriate blood samples for estradiol, free testosterone, free testosterone (percent), follicle stimulating hormone, luteinizing hormone, prolactin, progesterone, sex hormone binding globulin, total testosterone, and dehydroepiandrosterone sulfate are collected.

[0556] Urine is collected for measuring specific gravity, glucose, ketones, bilirubin, pH, urobilinogen, leukocytes, nitrites.

[0557] The Intrinsa® patch is removed (as appropriate).

[0558] Safety assessment is recorded.

[0559] Clinical Assessments

[0560] Screening and Covariate Questionnaires are used for clinical assessments.

BDI

[0561] To index the current level of depressive symptoms, the 21-item BDI-II is administered (Beck, Steer, & Brown.

1996), Dutch adaptation (Van der Does, 2002). The range for the BDI total score is 0-63, with higher scores indicating more depressive symptoms.

MMQ

[0562] The Maudsley Marital Questionnaire (MMQ; Crowe, 1978) is a 20-item self-report instrument measuring dissatisfaction with the general relationship, with the sexual relationship, and with life in general. The MMQ has shown good reliability and validity. The psychometric qualities of the Dutch version of the MMQ were also found to be satisfactory (Arrindell, Boelens, & Lambert. 1983). Higher scores represent larger dissatisfaction.

FSFI

[0563] The level of the woman's sexual functioning is assessed by the Female Sexual Function Index (FSFI; Rosen, Brown, Heiman, et al. 2000). The FSFI® is a self-administered questionnaire that consists of 19 questions. The scale contains six domains: desire, arousal, lubrication, orgasm, satisfaction, and pain. The range for the total score is 2-36, with lower scores representing worse sexual function. The psychometric quality of the FSFI is satisfactory (Wiegel, Meston, & Rosen. 2005). Based on a Dutch sample consisting of approximately 350 women with and without sexual complaints, the internal consistency and stability of the FSFI were found to be satisfactory-to-good. The FSFI's ability to discriminate between sexually functional and dysfunctional women was excellent as was the ability to predict the presence or absence of sexual complaints (ter Kuile, Brauer, & Laan, 2006).

FSDS-R

[0564] The woman's level of personal distress due to sexual dysfunction is assessed by the Female Sexual Distress Scale-Revised (FSDS-R®; Derogatis, Clayton, Lewis-D'Agostino, et al. 2008).

[0565] The items inquire about negative feelings and problems that are bothersome or cause distress during the past 30 days. Reliability and validity of the FSDS® (12-item version) has been evaluated in different samples of sexually functional and dysfunctional women. For the FSDS®, results indicated a unidimensional factor structure, a high degree of internal consistency, and test-retest reliability. The FSDS® showed a high degree of discrimination between sexually dysfunctional and functional women in each of its three validation studies. Results in a Dutch sample supported the unidimensional structure of the FSDS and its reliability and psychometric validity (ter Kuile, Brauer, & Laan, 2006). An additional question (question 13) has been added to the validated FSDS®. This question is about distress specifically related to sexual desire. The maximum total score of the FSDS-R® indicating the maximum level of sexual distress is '52'. Both the FSDS-R® total score and the Question 13 score alone will be analyzed.

Index of Sexual Satisfaction (ISS)

[0566] The woman's level of sexual satisfaction as assessed by the Index of Sexual Satisfaction (ISS; Hudson, Harrison, & Crosscup. 1981). This 25-item questionnaire asks subjects to evaluate various aspects of their sexual relationship, leading to a sum score that can range between 0 and 100. Higher scores correspond to greater sexual satisfaction. This measure

has been shown to have good face, convergent, and discriminant validity with various samples. Example items are “I feel that my partner enjoys our sex life,” “I think that sex is wonderful,” and “My partner is sexually very exciting.” For the purpose of the present study, the ISS is translated into Dutch.

SDI-II

[0567] The level of the woman’s sexual desire is assessed by the Sexual Desire Inventory-II (SDI-II; Spector, Carey, & Steinberg, 1996). The SDI-II consists of two seven-item self-report scales: the Dyadic Sexual Desire scale, which measures an individual’s desire for sexual activity with a partner, and the Solitary Sexual Desire scale, which measures an individual’s desire for autoerotic sexual activity. The two subscales were internally consistent (Cronbach’s α : Dyadic scale=0.86; Solitary scale=0.96).

Sexual Excitation/Sexual Inhibition (SESII-W)

[0568] The Sexual Inhibition/Excitation Inventory for women (SESII-W; Graham, Sanders, & Milhausen, 2006) is used to assess individuals’ propensity for sexual excitation and sexual inhibition. It consists of 36 items, referring to stimulus situations that could affect sexual inhibition and sexual excitation or to general statements about arousability and inhibition. The instructions ask women to report what would be the most typical reaction now or how they think they would respond if the item does not apply to them. Items are rated on a 4-point Likert-rating scale, from “strongly disagree” to “strongly agree.” The SESII-W has eight lower-order factors, which in turn load on two higher-order factors, Sexual Excitation and Sexual Inhibition. The questionnaire shows good test-retest reliability and convergent and discriminant validity and Sexual Excitation and Sexual Inhibition appear to be relatively independent factors. The list is already in use in the Netherlands, but psychometric properties have not been investigated yet.

[0569] Efficacy Pharmacodynamic Testing

Computer Testing

Single Target Implicit Association Task (StIAT):

[0570] Conform Wigboldus et al. (2005), the StIAT used in this study is designed to assess subjects’ affective associations with sexual stimuli (Brauer, van Leeuwen, Janssen, et al. submitted). Subjects are instructed to classify pictures portraying sexual acts (i.e., target stimuli) and words representing “positive” or “negative” meanings (i.e., attribute stimuli) to the appropriate superordinate category (i.e., “sex”, “positive”, “negative”) as quickly as possible by pressing only a left or right response key on a keyboard. These labels used for these categories (sex, positive, negative) are continuously visible on the computer screen. The StIAT consists of a combination of practice and experimental blocks (see Greenwald, McGhee & Schwartz, 1998 for detailed methodology). The experimental blocks consist of one ‘incongruent’ and one ‘congruent’ block of trials. In the incongruent block, “sex” and “negative” are mapped on a single key and “positive” on the other, while in the congruent block, “sex” and “positive” are mapped on the same key and “negative” on the other. The difference in reaction times between the two experimental blocks is assumed to reflect whether sex is associated more strongly with either positive or negative. Faster responses in

the congruent block (compared to the other block) reflect stronger associations between positive and sex, and faster responses in the incongruent block reflect stronger associations between negative and sex. The target-attribute combinations that share response keys (i.e., block order), and left or right key response requirements are counterbalanced. Each critical block consists of 40 trials of which responses were divided equally over the two response keys. The target category consists of 5 exemplar stimuli of sexual images from the International Affective Picture System (IAPS; Center for the Study of Emotion and Attention, 1995), with the following numbers: 4800, 4652, 4658, 4659, and 4672. The attribute categories consists of 20 generally positive and 20 generally negative words (Dotsch & Wigboldus, 2008; Dotsch, Wigboldus, Langner et al. 2008), thus reflecting more global affective associations with sex. These words were controlled for length and frequency. With respect to the validity, the StIAT’s strength lies in high effect sizes due to double opposing categories often leading to slower reaction times (the categorization decision requires effort as there are several possibilities to consider).

Picture Association Task (PAT)

[0571] This task, developed by van Leeuwen and Macrae (2004), is based on the Affective Priming Task (e.g., Bargh, Chaiken, Gendler, et al. 1992; Fazio, Sanbonmatsu, Powell, et al. 1986; Hermans, De Houwer, & Eelen, 1994) where target words are preceded by another word or image that influences the categorization speed of the target word. In the PAT, however, target words and images appear simultaneously. In the PAT used in this study, subjects are presented with positive or negative words superimposed on either sexual or neutral pictures (Brauer, van Leeuwen, Janssen, et al. submitted). They are instructed to categorize the words as fast as possible as either positive or negative by pressing one of two computer keys. Subjects are further instructed to focus on the words that appeared on the screen and not to attend to the background images as these are of no importance for the task and the categories to which the pictorial stimuli belong (sex, neutral) are not explained. Thus, the PAT captures the unintentional influence of the affective value of the pictorial background stimuli on task performance. The time to select the correct response to the words (positive or negative) is influenced by the match between the valence of the word and the valence of the background image (sex or neutral), thereby revealing indirectly the valence of the picture for the subjects. The word categories consist of 10 positive words and 10 negative words. Whereas for the StIAT general positive and negative words are selected (e.g., peace, respect, war, hate), the PAT consists of positive and negative words that are applicable to a sexual situation, but that do not exclusively refer to sexual experiences (e.g., enjoyable, wonderful, dirty, disgusting) in order to create a conceptual overlap between the content of the words and the content triggered by the sexual pictures. These words are taken from a pilot study in the Netherlands in which female subjects (N=20) were asked to indicate on a 7-point Likert scale for each positive and negative word how well it described a positive or a negative sexual situation, respectively (Brauer & Laan, 2008). The words appear at one of four randomized locations on the picture to avoid expectation-related responses and to make sure subjects would move their eyes over the image. The sexual pictures were taken from another study on implicit associations with sexual stimuli in women with dyspareunia

(Brauer, de Jong, Huijding et al., 2009). These pictures display a variety of sexual acts (e.g., kissing, cunnilingus, fellatio, coitus). Based on each sexual picture, a control picture was created by scrambling the sexual image, leaving a neutral stimulus. All pictures are standardized to 600×480 pixels and matched for brightness and contrast. Each stimulus remains on the screen until subjects make a decision or until 3,000 ms has elapsed. After 10 practice trials, 80 experimental trials are presented. Each word is paired randomly with a sexual picture and a neutral picture, resulting in four different combinations each presented 20 times: positive words and sexual images, negative words and sexual images, positive words and neutral images, negative words and neutral images. The order of presentation of the trials is counterbalanced within, and response key mappings (i.e., positive/negative or negative/positive) are counterbalanced across subjects. The computer records the accuracy and latency of each response. With respect to validity, the strength of the PAT is that it is not sensitive to a possible interpretation bias due to the need to attend to the different stimulus categories at the same time, as is the case in the stIAT.

Dot Probe Task (DOT)

[0572] The dot-probe task (DOT) assesses attentional preference for sexual and neutral visual stimuli. In this task, subjects are shown two images side by side on a computer screen for 500 ms. When the two images disappear, a target stimulus represented by a small dot appears in the place of one of the images. Subjects are asked to indicate the location (side) of the dot. Mean RTs are calculated for three categories: 1) neutral neutral 2) neutral sex with the dot under neutral 3) neutral sex with the dot under sex. If reaction times are faster when the dot appears in the place of a certain class of stimuli this indicates an attentional bias towards this class of stimuli.

Psychophysiological Testing

Genital Response (VPA)

[0573] Psychophysiological testing consists of assessment of genital response (vaginal pulse amplitude) and subjective sexual arousal during sexual to self-induced erotic fantasy (3 min), a low-intensity erotic film clip (5 min), and a high-intensity erotic film clip (5 min) (Laan et al., in preparation). The erotic conditions are separated by variable interstimulus intervals during which subjects complete a concentration task (simple arithmetic problems) to allow for return-to-baseline. The erotic stimulus testing is preceded by a 8 min neutral film to establish baseline levels. VPA is measured using a vaginal photoplethysmograph developed by Bert Molenkamp (Technical Support, Department of Psychology, University of Amsterdam) based on instruments initially developed by Sintchak and Geer (1975). The light source (3 mm LED, $\lambda=620$ nm) and optical sensor (Texas Instruments TSL250) are produced in batches of 100, resulting in all photoplethysmographs used in this study having nearly equal electronic characteristics. A signal-conditioning amplifier separates the VPA from the direct current component using a 12 dB/octave, 0.7-Hz filter. Additional filtering for VPA is 24 dB/octave, 0.4 Hz high-pass. The VPA signal is digitalized at 100 Hz with a Keithley KPCI3107 A/D converter, running on a Windows 2000 PC system. Depth of the probe and orientation of the light source is controlled by a device (a 9-x2-cm FDA-approved perspex plate) attached to the cable within 5 cm of the

optical sensor. Subjects are instructed to insert the probe until the plate touched their labia. The probe and plate are sterilized according to standard department protocol.

Sexual Feelings and Affect (SAQ)

[0574] Prior to and immediately after erotic stimulus subjects fill out a questionnaire measuring sexual feelings and affect during sexual stimulation, consisting of 5 scales: sexual arousal (Cronbach's $\alpha=0.87$); genital sensations (Cronbach's $\alpha=0.96$); sensuality (Cronbach's $\alpha=0.73$); positive affect (Cronbach's $\alpha=0.93$); and negative affect (Cronbach's $\alpha=0.65$). Each question is preceded by the sentence: "During the film, I felt:" after which a positive, negative, physical or sexual experience is described, for instance, pleasant; worried; genital pulsing or throbbing; sexually aroused. The items are measured on a 1 (not at all) to 7 (intensely) scale.

Acute Female Sexual Desire (AFSDQ)

[0575] Prior to and following psychophysiological testing subjects fill out the Acute Female Sexual Desire Questionnaire (Laan, Heiman, unpublished). This questionnaire assesses sexual interest in erotic stimuli and has shown to discriminate between women with acquired HSDD and sexually functional controls (Laan et al., in preparation).

[0576] Statistical Methodology

[0577] Calculation of Pharmacokinetic Parameters

[0578] C_{min} , C_{max} , and t_{max} are taken from the actual measured values. Values are determined relative to the testosterone administration time in treated subjects.

[0579] Area under the concentration curve (AUC) are estimated for the 0 to 24 hour time interval, as well as the BID dosing intervals, using the trapezoidal rule.

[0580] PK evaluations after Day 1 evening dose for testosterone gel formulations of the invention and placebo and Day 3 evening dose for testosterone gel formulations of the invention, placebo and Intrinsa® patch (which was applied on Day 1)—AUC, concentrations of total and free testosterone, DHT, estradiol, SHBG. Analyses of C_{avg} , C_{min} , C_{max} , t_{max} , AUC_{0- τ} , PTF, and PTS. C_{avg} are calculated for the 12 hour period as well as τ when appropriate. For subjects on Intrinsa®, a 24 hour calculation is performed.

[0581] The average concentration in the dosing interval (C_{avg}) is calculated from the AUC using the following formula: $C_{avg}=AUC_{0-\tau}/\tau$, with τ =dosing interval time.

[0582] Peak Trough Fluctuation (PTF) and Peak Trough Swing (PTS) are calculated as follows:

$$PTF=(C_{max}-C_{min})/C_{avg}$$

$$PTS=(C_{max}-C_{min})/C_{min}$$

[0583] Percent time that the plasma testosterone concentration is above, within, and below the reference range of 10 to 70 ng/dl, is calculated.

[0584] Statistical Analysis of Pharmacodynamic Data stIAT:

[0585] Incorrect responses are excluded from analyses. In addition, RTs shorter than 300 ms or longer than 3000 ms are excluded from analyses. With respect to the stIAT data, Wigboldus, Holland & van Knippenberg (2005) is followed in that, for each subject, the median response latency of the correct responses to the attribute items in congruent and incongruent blocks is used. Following this, median reaction times of the two experimental blocks are subtracted from one

another to obtain a stIAT effect (i.e., stIAT effect=median (Sex/Negative)–median (Sex/Positive)). Negative stIAT effects indicate relatively stronger negative associations with sexual stimuli.

[0586] The stIAT effect is analyzed with an analysis of variance with fixed factor treatment, group (HSDD and SA) and the interaction treatment by group. The contrasts are calculated within the model.

PAT:

[0587] Median response latencies of the correct responses are calculated, following van Leeuwen and Macrae (2004). To correct for baseline reactions to positive and negative words, difference scores are calculated by subtracting RTs for neutral words superimposed on sexual pictures from positive words superimposed on sexual pictures. The same is done for negative words superimposed on sexual and neutral pictures (i.e., Sex/+RT (sex/positive words)–RT (neutral/positive words) and Sex/–RT (sex/negative words)–RT (neutral/negative words). Sex/+<Sex/–=automatic positive associations with sex).

[0588] The two PAT variables (RT positive and RT negative) are analyzed with an analysis of variance with fixed factors treatment, group (ANOR) and group by treatment. The contrasts will be calculated within the model.

DOT:

[0589] For each subject the difference between mean RT for the category neutral sex with the dot under sex and the mean RT for the category neutral sex with the dot under neutral is calculated to obtain a DOT effect (i.e., DOT effect=mean neutral sex with dot under neutral–mean neutral sex with dot under sex). Higher DOT scores indicate relatively stronger attention for sexual stimuli.

[0590] The DOT effect is analyzed with an analysis of variance with fixed factor treatment, group (ANOR) and the interaction treatment by group. The contrasts are calculated within the model.

VPA:

[0591] After VPA artefact deletion, done by a computer program developed Bert Molenkamp (Technical Support, Department of Psychology, University of Amsterdam), peak-to-trough amplitude is calculated for each remaining pulse. VPA is averaged every 30 seconds during several conditions: neutral film (8 min), self induced erotic fantasy (3 min), low intensity erotic film clip (5 min) and high intensity erotic film clip (5 min). All conditions are offered twice: once 0.5 hours after application of the nasal gel and once 4.5 hours after application of the nasal gel.

[0592] VPA during the erotic fantasy, the low intensity film and the high intensity film are analyzed separately and the different moments (0.5 hours after and 4.5 hours after dosing) are also be analyzed separately, resulting in 6 analyses. VPA during a condition and a moment is analyzed with a mixed model analysis of variance with fixed factors treatment, group (ANOR), time, group by treatment, treatment by time and random factor subject and the average VPA score during the neutral film as covariate. Contrasts are calculated within the model.

SAQ:

[0593] For each of the five SAQ scale mean response during a condition and a moment are analyzed with an analysis of variance with factors treatment, group (ANOR) and group by treatment, with the score prior to sexual stimuli as covariate. Contrasts are calculated within the model.

AFSDQ:

[0594] ASFDQ score after erotic stimulation during a moment is analyzed with an analysis of variance with factors treatment, group (ANOR), and group by treatment; and the score prior to sexual stimuli as covariate. Contrasts are calculated within the model.

[0595] If necessary to meet requirements for analysis of variance data is log-transformed. Results are back-transformed and reported as % change.

[0596] Graphs of least square means estimates over time by treatment are presented with error bars indicating the upper and lower 95% confidence interval for the highest and lowest profile respectively. Least square means of the contrasts are tabulated.

[0597] If analyses are not feasible according to the described models with the given data, analyses are adjusted. If considered useful extra exploratory analyses are conducted.

[0598] Statistical Analysis of Safety Data

[0599] Nasal Tolerance: Nasal tolerance data is presented in summary tables. No statistical analysis will be performed.

[0600] Vital Signs and Clinical Laboratory Parameters:

[0601] A table summarizing all laboratory test values and changes from Baseline is presented for each treatment group. In case parameters are $\pm 20\%$ of their reference range, the clinical significance of these findings are evaluated.

[0602] The results of this analysis is presented in FIGS. 2, 3 and 7-8. FIGS. 3, 9-11 show or compare the results between the effects of the testosterone gel nasal formulations of the invention on subjects diagnosed with anorgasmia or HSDD.

Example 9

Pharmacokinetic ("PK") and Pharmacodynamic ("PD") Study Concerning 53 Anorgasmia Women and the Three Different Testosterone Bio-Adhesive Gel Formulations of the Invention

0.15%-, 0.45% and 0.6% Testosterone by Weight of the Gel Formulation, as Reported in Examples 1-5

Objective:

[0603] As shown in FIG. 14, this PK and PD study is to assess the serum testosterone pharmacokinetic profile and the pharmacodynamic response measuring Vaginal Pulse Amplitude ("VPA") following a single dose administration of each of the testosterone bio-adhesive gel formulations of the invention (0.15%-, 0.45% and 0.6% testosterone by weight of the gel formulation, as reported in Examples 1-5), as compared to placebo, in women with anorgasmia.

Method:

[0604] A total of 12 women with anorgasmia (n=12) are included in this placebo and active comparator PK and PD study. Each women receives four different single intranasal doses of 100 μ l per nostril administered via a unit-dose

syringe on four different days (that is, each of the 12 women in this study receives a TBS-2 high dose (1.2 mg-0.6% by weight testosterone—0.6 mg/100 μ l/nostril), a TBS-2 medium dose (0.9 mg-0.45% by weight testosterone—0.45 mg/100 μ l/nostril) or a TBS-2 low dose (0.3 mg-0.15% by weight testosterone—0.15 mg/100 μ l/nostril), and placebo TBS-2 (anorgasmia cohort). Frequent PK serum ampules are collected from each woman during the first 12 hours after intranasal dose administration. Initial pharmacodynamic efficacy is explored using vaginal pulse amplitude (VPA). VPA is a measure of blood flow to the vagina (engorgement). Safety is monitored throughout the PK and PD study.

Results:

[0605] The PK results show that there is an increase in plasma testosterone levels with increasing dose levels. See FIG. 14. Mean concentrations of plasma testosterone 0-12 hours after dosing are: (a) TBS-2 high dose (1.2 mg-0.6% by weight testosterone—0.6 mg/100 μ l/nostril)—about 70 ng/dL after about the first 100 minutes of administration, about 50 ng/dL after about the first 250 minutes of administration, and about 40 ng/dL after about 350 minutes following administration and thereafter; (b) TBS-2 medium dose (0.9 mg-0.45% by weight testosterone—0.45 mg/100 μ l/nostril)—about 55 ng/dL after about the first 25 minutes of administration, about 35 ng/dL after about the first 250 minutes of administration, and about 35-30 ng/dL after about 350 minutes following administration and thereafter; and (c) TBS-2 low dose (0.3 mg-0.15% by weight testosterone—0.15 mg/100 μ l/nostril)—about 28 ng/dL after about the first 100 minutes of administration, about 23 ng/dL after about the first 250 minutes of administration, and about 20 ng/dL after about 350 minutes following administration and thereafter.

[0606] The testosterone C_{max} and C_{avg} for testosterone following single dose administration for each of the three TBS-2 dosage strengths do not exceed the normal testosterone serum level in women (3-80 ng/dL).

[0607] For all TBS-2 dosage strengths, the testosterone level returns to baseline following administration of the single dose.

[0608] As to the PD aspect of the study, there are favorable statistically significant differences in VPA response in the women for each of the TBS-2 dosage strengths vs. placebo at 0.5 hours and at 4.5 hours post-dose.

Conclusion:

[0609] It is presently believed that the TBS-2 nasally applied testosterone bio-adhesive gels of the preset invention (i) may be uniquely taken on a prn basis, i.e., on demand, (ii) have an ideal safety profile, i.e., there appears to be no androgen-related side effects, there is a low testosterone drug load, and (iii) present no risk of testosterone transference.

Example 10

Vibrotactile Stimulation Study ("VTS") of 56 Women with Anorgasmia

Objective:

[0610] To evaluate the effect of a single dose of high dose TBS-2 (1.2 mg-0.6% by weight testosterone—0.6 mg/100 μ l/nostril, as reported in Examples 1-5) on orgasm at 0.5, 2.0, 4.0 and 8.0 hours post-dose administration in 56 women with

anorgasmia. Another objective of this study is to evaluate the time to orgasm and the quality of the orgasm following TBS-2 intranasal administration. Further, an objective of this VTS study is to determine the effect of high dose TBS-2 on arousal, sensuality and genital stimulation and to assess safety. See FIG. 15.

Method:

[0611] This VTS study is a single-center, randomized, single-blind, placebo-controlled, five-arm parallel group study using vibrotactile stimulation that is combined with visual sexual stimulation (n=56). See FIG. 15.

[0612] In accordance with this VTS study protocol, each woman is administered an intranasal single high-dose of TBS-2 (1.2 mg-0.6% by weight testosterone—0.6 mg/100 μ l/nostril).

[0613] The demographics for this VTS study are, see FIG. 15:

[0614] 59 women randomized, 56 completed the study

[0615] All 3 drop-outs due to protocol violations

[0616] Average age 27.8 years

[0617] 87.5% Primary Anorgasmia, 12.5% Secondary Anorgasmia

[0618] All randomized subjects met entrance criteria for Marital Quality and Depression

[0619] 3% of patients (3 out of 97) excluded due to orgasm at Visit 2

Results:

[0620] As shown in FIG. 16, more women report, treated with high dose TBS-2, report orgasms, as compared to placebo. In fact, during the 4 weeks post-treatment phase, women who are treated with high dose TBS-2 8 report a total of 8 orgasms, as compared to no orgasms for those women who receive placebo. With respect to the treatment phase, women who are treated with high dose TBS-2 8 report a total of 4 orgasms, as compared to 2 orgasms for those women who receive placebo. See FIG. 16.

[0621] Additional VTS study results and findings include:

[0622] The time to orgasm ranges from 12.17 minutes to 18.22 minutes following high dose TBS-2 administration;

[0623] The orgasms by women, who are administered TBS-2, are reported as more pleasant and more intense as compared to the orgasms by those women who are administered placebo;

[0624] More TBS-2 women report high arousal compared to Placebo (83.3% vs. 16.7%). See FIGS. 17 and 18;

[0625] TBS-2 women report more sexual desire (AFSDQ Scores). See also FIGS. 17 and 18;

[0626] Positive response to stimulation more pronounced for TBS-2. See FIG. 18;

[0627] As reported in Example 9, there is statistically significant differences in VPA between TBS-2 high, medium and low dose and Placebo (mean change from baseline);

[0628] Total testosterone levels are increased to upper end of normal (mean about 66.7 ng/dL);

[0629] Mean free testosterone levels are about 6.35 pg/mL (chronic treatments achieve about 3.1-4.0 pg/mL);

[0630] Other Findings—Patient Feedback**[0631]** (a) Women prefer home setting**[0632]** (i) thoughts about hospital setting, experimenter, are distracting**[0633]** (ii) still a little tense, because of the setting**[0634]** (iii) home is a better setting**[0635]** (iv) at home, more relaxed**[0636]** (v) a little limited in movement due to position**[0637]** (b) Partner Involvement Important to some women**[0638]** (i) with real life partner, it would be better**[0639]** (ii) felt guilty he is (partner) is not here**[0640]** (iii) nicer with a man

Safety:

[0641] No Serious Adverse Events**[0642]** Total of 18 Adverse Events reported**[0643]** (i) mild and resolved by end of study**[0644]** (ii) 5 Not Related to study medication**[0645]** (iii) 3 of unknown etiology (Possibly Related)**[0646]** (iv) no association with active treatment and adverse events**[0647]** (v) placebo had 50% of women report adverse events vs. 19.1% for TBS-2**[0648]** No difference in endocrinology results (SHBG, Albumin, Hemoglobin)

Conclusion

[0649] Adequate clitoral stimulation alone is not sufficient to treat anorgasmia**[0650]** The data from Examples 10 and 11 point to the success of TBS-2 pharmaceutical intervention in addition to effective stimulation**[0651]** TBS-2 data indicates a positive response between 2 and 8 hours post-dose**[0652]** TBS-2, when administered prn, is believed to elicit and enhance sexual response in women**[0653]** TBS-2 is believed to maintain total and free testosterone levels in the normal range**[0654]** TBS-2 is believed to be safe and none of the adverse events commonly observed with chronic testosterone treatments are observed**[0655]** Comfortable environment (home setting) and partner interaction may play a role in achievement of orgasm

Example 11

1. Title

An Open-Label, Single and Multiple-Application of
Intranasal Testosterone Gel (TBS-2) in Healthy
Pre-Menopausal Female Subjects at Three Dose
Levels

2. Synopsis

[0656] The primary objective is to assess the bioavailability of total testosterone through pharmacokinetic (PK) profiles obtained following single administration of an intranasal application of TBS-2 at doses of 600 µg, 1200 µg, and 1800 µg, and multiple administration of 1200 µg of TBS-2 given three times a day (t.i.d.) for 3 days. TBS-2 is a bioadhesive intranasal testosterone gel.

[0657] The secondary objectives are to assess the bioavailability of free testosterone, dihydrotestosterone, sex hormone-binding globulin (SHBG), and estradiol through PK profiles obtained following single administration of an intranasal application of TBS-2 at doses of 600 µg (0.24%), 1200 µg (0.48%), and 1800 µg (0.72%), and multiple administration of 1200 µg (0.72%) TBS-2 given for 3 days (t.i.d. for the first 2 days, and once in the morning of the third day) and to assess the safety of TBS-2

[0658] A. Methodology

[0659] This was a phase 1, single-center, randomized, open-label parallel-group study designed to evaluate the safety, tolerability, and PK of TBS-2 in healthy, normal-cycling adult women. Subjects were randomly assigned on a 1:1:1 basis to 1 of 3 treatment groups (Cohort 1, Cohort 2, or Cohort 3) during Period 1 and were administered a single dose of an intranasal application of TBS-2 at doses of 600 µg, 1200 µg, or 1800 µg (single doses of 300 µg, 600 µg, and 900 µg per nostril). At the end of Period 1, a total of 8 subjects sampled from these 3 cohorts, who were willing and able to continue with the multiple-dose portion of the study were selected to participate in Period 2. During Period 2, subjects were administered 1200 µg TBS-2 (600 µg per nostril) t.i.d. for 2 days and once on the morning of the third day. Subjects were screened (Visit 1) for eligibility up to 3 weeks prior to dosing in Period 1, and were admitted to the Clinical Research Unit (CRU) at 0700 hours on the day prior to dosing (Visit 2, Day 1). On Day 2, subjects were administered a single dose of TBS-2 and remained in the CRU for 72 hours post-dose (Day 4) for safety monitoring and PK assessments. During Period 2, subjects were admitted to the CRU at 0700 hours on the day of dosing (Visit 3, Day 1). On Days 1 and 2, subjects were administered TBS-2 at 0800 hours (±30 minutes), 1600 hours (±30 minutes), and 2400 hours (±30 minutes). On Day 3, subjects were administered TBS-2 at 0800 hours (±30 minutes).

[0660] During Period 1, blood samples for determination of baseline testosterone (free and total), SHBG, dihydrotestosterone (DHT), and estradiol concentration were collected on Day 1 at 0745 hours and then at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, and 23.5 hours relative to an 0800 hour clock time. Blood samples for determination of testosterone (free and total), SHBG, dihydrotestosterone, and estradiol plasma concentration were collected on Day 2 (15, 30, and 45 minutes and 1, 1.5, 2, 4, 6, 8, 12, 16, and 20 hours post-dose) and Day 3 (24, 32, 40, and 48 hours post-dose) during the confinement period.

[0661] During Period 2, a blood sample for baseline testosterone (free and total), SHBG, DHT, and estradiol concentration was collected at 0745 hours (i.e., 15 minutes prior to study drug administration). Blood samples for determination of testosterone (free and total), SHBG, dihydrotestosterone, and estradiol concentration were collected on Day 1 (pre-dose [15 minutes prior to dosing] and at 1545 and 2345 hours), Day 2 (1545 and 2345 hours), Day 3 (15, 30, and 45 minutes and 1, 1.5, 2, 4, 6, 8, 12, 16, and 20 hours), and Day 4 (24, 32, 40, and 48 hours) during the confinement period.

[0662] Other assessments performed during the study included the monitoring of adverse events (AEs), clinical laboratory evaluations (chemistry [including hormone profile], hematology, and urinalysis), vital signs assessments (systolic and diastolic blood pressure [BP], heart rate [HR], respiratory rate [RR], and body temperature), and physical examinations. In addition, otorhinolaryngological examina-

tion findings, 12-lead electrocardiogram (ECG) readings, medical history, and concomitant medication use were recorded.

[0663] B. Number of Subjects (Planned and Analyzed)

[0664] Planned: A total of 24 subjects were planned to be enrolled.

[0665] Enrolled: A total of 24 subjects were enrolled and randomly assigned to treatment in Period 1: 8 subjects in Cohort 1, 8 subjects in Cohort 2, 8 subjects in Cohort 3. A total of 8 subjects sampled from these 3 cohorts, who were willing and able to continue with the multiple-dose portion of the study, were selected to participate in Period 2.

[0666] Analyzed: All 24 subjects were included in the safety analyses and 24 subjects were included in the PK analyses.

[0667] C. Diagnosis and Main Criteria for Inclusion

[0668] Healthy, normal-cycling women between the ages of 18 and 40 years (inclusive) who were premenopausal, had a body mass index (BMI) of 18.5 to 35 kg/m² (inclusive), met all of the inclusion and none of the exclusion criteria, and provided informed consent were included in the study.

[0669] D. Test Product, Dose and Mode of Administration, Batch Number

[0670] The TBS-2 used in this study was an intranasal testosterone gel supplied in prefilled dispensers with 0.24% testosterone gel to deliver a single intranasal dose of 300 µg of testosterone per nostril (Cohort 1), 0.48% testosterone gel to deliver a single intranasal dose of 600 µg of testosterone per nostril (Cohort 2 [single-dose] and Multidose group), and 0.72% testosterone gel to deliver a single intranasal dose of 900 µg of testosterone per nostril (Cohort 3). The lot numbers of TBS-2 drug substance used in this study were IMP11008, IMP11009 and IMP11010.

[0671] The compositions of the three different concentrations of the drug product to be used in this clinical trial are provided in the CMC Section below and in Tables 3.2.P.1-1-3.

[0672] E. Duration of Treatment

[0673] The study involved 1 period for Cohorts 1, 2, and 3 and the duration of individual subject participation from the start of screening until the post-study visit, was approximately 25 days. This study involved 2 periods totaling 30 to 36 days for the Multi-dose group.

[0674] F. Criteria for Evaluation

[0675] Safety: Safety was assessed throughout the study and included the monitoring of AEs, clinical laboratory evaluations (chemistry [including hormone profile], hematology, and urinalysis), vital signs, and 12-lead ECGs. Physical and otorhinolaryngological examinations were performed and medical history and concomitant medication use were recorded.

[0676] Pharmacokinetics: Whole blood samples for determination of plasma concentrations of testosterone (free and total), SHBG, DHT, and estradiol were collected at specified time points. Actual sampling time points were recorded and used for PK calculations. Pharmacokinetic parameters for testosterone (free and total), SHBG, DHT, and estradiol were calculated by standard non-compartmental methods for all subjects as data permitted. The PK parameters evaluated for plasma concentrations of testosterone (free and total), SHBG, dihydrotestosterone, and estradiol following the single-dose cohorts (Cohorts 1, 2, and 3) included area under the plasma concentration time curve from time zero to the last measurable concentration time point (AUC_{0-t}), area under the plasma concentration time curve from time zero to infinity ($AUC_{0-\infty}$),

maximum concentration observed after dosing (C_{max}), time of observed C_{max} relative to the time of dosing (t_{max}), terminal elimination rate constant (λ_z), and elimination half-life ($t_{1/2}$). The PK parameters evaluated for plasma testosterone (free and total), SHBG, dihydrotestosterone, and estradiol concentrations following the multiple-dose cohort included area under the concentration-time curve from time zero to the dosing interval ($AUC_{0-\tau}$, where $\tau=8$ hours), C_{max} , t_{max} , minimum concentration over a dosing interval during multiple dosing (C_{min}), pre-dose concentration determined immediately before a dose at steady state (C_{pd}), average steady-state concentration (C_{avg}), % peak to trough fluctuation (PTF), and % peak to trough swing (PTS).

[0677] G. Statistical Methods

[0678] This study evaluated the PK properties as well as the safety and tolerability of TBS-2. Power calculations were not performed. The sample size for this study was not determined on the basis of statistical hypothesis testing. Based on typical, early-stage PK studies, groups of 8 subjects per cohort provided adequate clinical information to satisfy the objectives of the study. Data were summarized by using descriptive statistics (sample size, mean, median, standard deviation [SD], minimum, and maximum) for each of the safety variables by treatment group and overall. Data from all visits during the study were displayed in the data listings.

[0679] Concentration-time data for 5 analytes (testosterone [total and free], SHBG, dihydrotestosterone, and estradiol) were determined by a validated assay method and PK parameters were calculated. Actual sampling time points were recorded and used in calculation of the actual elapsed time from dose to sample for PK calculations. As each administration was to each of the nostrils, the time of dosing was the time of the first nostril administration. Baseline analyte concentrations from the 24-hour pre-dose profile were subtracted from the time-matched analyte concentrations following dose administration before calculation of the PK parameters.

[0680] Individual PK parameters were estimated for each subject's profile in the PK population by using WinNonlin (Pharsight Corporation) and were displayed in the data listings. Data were summarized by using descriptive statistics (mean, SD, % coefficient of variation [CV], confidence interval (CI), median, minimum, and maximum) and are presented by treatment group. Geometric means were included for AUC and C_{max} estimations and were included for some other PK parameters. By using Generalized Linear Model (GLM) procedure in SAS®, an analysis of variance (ANOVA) was performed on natural logarithmic (ln) transformed parameters AUC_{0-t} , $AUC_{0-\infty}$, $AUC_{0-\tau}$, C_{avg} , and C_{max} and on untransformed parameters $t_{1/2}$, and λ_z at the significance level of 0.05. The intrasubject CV was calculated for AUC_{0-t} , $AUC_{0-\infty}$, $AUC_{0-\tau}$, and C_{max} by using the ANOVA residual error.

[0681] Dose linearity following single-dose administration (Period 1) was assessed after natural-log transformation for the AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} .

[0682] The following Period 1 comparisons for PK parameters were made:

[0683] Comparison 1: 600 µg (0.24%) TBS-2 versus 1200 µg 0.48% TBS-2;

[0684] Comparison 2: 600 µg (0.24%) TBS-2 versus 1800 µg 0.72% TBS-2;

[0685] Comparison 3: 1200 µg (0.48%) TBS-2 versus 1800 µg 0.72% TBS-2.

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4. Glossary of Abbreviations and Terms

- [0734] AE adverse event
- [0735] ALT alanine transaminase
- [0736] ANOVA analysis of variance
- [0737] AST aspartate transaminase
- [0738] AUC area under the plasma concentration time curve
- [0739] AUC_{0-∞} AUC from time zero to infinity
- [0740] AUC_{0-t} AUC from time zero to the last measurable concentration time point
- [0741] AUC_{0-τ} AUC from time zero to the dosing interval (where τ=8 hours) during multi-dose period
- [0742] AUC₀₋₈ AUC from time zero to 8 hours during single-dose period
- [0743] BMI body mass index
- [0744] BP blood pressure
- [0745] BUN blood urea nitrogen
- [0746] C_{avg} average steady-state concentration

- [0747] CFR Code of Federal Regulations
- [0748] CI confidence interval
- [0749] CK creatine kinase
- [0750] CL Clearance
- [0751] C_{max} maximum concentration observed after dosing
- [0752] C_{min} minimum concentration over a dosing interval during multiple dosing
- [0753] ConcBase baseline concentration
- [0754] ConcBC baseline corrected concentration
- [0755] ConcBLQ Active dose concentrations corrected for BLQ
- [0756] Cpd pre-dose concentration determined immediately before a dose at steady state
- [0757] CRF case report form
- [0758] CRU clinical research unit
- [0759] CS clinically significant
- [0760] CV coefficient of variation
- [0761] DHT Dihydrotestosterone
- [0762] ECG Electrocardiogram
- [0763] eCRF electronic case report form
- [0764] FDA Food and Drug Administration
- [0765] FSD female sexual dysfunction
- [0766] GCP good clinical practice
- [0767] GGT gamma-glutamyl transferase
- [0768] GLM Generalized Linear Model
- [0769] HbsAg hepatitis B surface antigen
- [0770] HCV hepatitis C virus
- [0771] HIV human immunodeficiency virus
- [0772] HR heart rate
- [0773] ICF informed consent form
- [0774] IRB institutional review board
- [0775] ln Logarithmic
- [0776] MedDRA Medical Dictionary for Regulatory Activities
- [0777] NCS not clinically significant
- [0778] OTC over-the-counter
- [0779] PD Pharmacodynamic
- [0780] PI Principal Investigator
- [0781] PK Pharmacokinetic
- [0782] PTF % peak to trough fluctuation
- [0783] PTS % peak to trough swing
- [0784] RR respiratory rate
- [0785] SAP statistical analysis plan
- [0786] SD standard deviation
- [0787] SHBG sex hormone-binding globulin
- [0788] $t_{1/2}$ elimination half-life
- [0789] t_{max} time of observed C_{max} relative to the time of dosing
- [0790] VPA vaginal pulse amplitude
- [0791] λ_z terminal elimination rate constant

5. Ethics

- [0792] A. Institutional Review Board (IRB)
- [0793] The study and any amendments were reviewed by the Institutional Review Board (IRB) for each center. Any subsequent protocol amendments or informed consent revisions were approved by the IRB before any changes were initiated.
- [0794] B. Ethical Conduct of the Study
- [0795] This study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and current good clinical practice (GCP) and in compliance with local regulatory requirements and 21 CFR 312.

[0796] C. Subject Information and Consent

[0797] The format and content of the subject information sheet and informed consent form (ICF) were agreed upon by the Principal Investigator (PI), the IRB, and Trimel Pharmaceuticals Corp. (hereafter referred to as Trimel). Each subject's written informed consent to participate in the study was obtained before any study specific procedures were performed. The PI or a medically qualified member of the study team provided the subject with a full explanation of the study drugs and manner of treatment allocation, the possible risks and benefits, and the compensation or treatment available in the event of study-related injury.

[0798] Subjects who agreed to participate in the study signed and dated an ICF. The ICF was also signed and dated by designated site personnel. The original and all amended signed and dated ICFs were retained at the study site, and copies of these forms were provided to the subject.

6. Investigators and Study Administrative Structure

[0799] This study was conducted at 1 investigative site in the United States. The PI at the investigative site was responsible for the validity and accuracy of the data supplied on the case report form (CRF). Delegation of authority for completion of the CRF was permitted, but the PI was responsible for its accurate completion and was required to sign the completed CRF that it was a true and accurate reflection of the subject's participation in the study.

[0800] The PI at the investigative site signed the protocol signature page. By signing this page, the PI agreed to conduct the study in accordance with the study protocol and also to comply with the requirements regarding the obligations of clinical investigators and all other pertinent requirements of applicable regulatory agencies.

7. Introduction

[0801] TBS-2 is developed for the treatment of anorgasmia.

[0802] A. Background

[0803] Anorgasmia is the second most frequently reported women's sexual problem after hypoactive sexual desire disorder. The Global Survey of Sexual Attitudes and Behaviors assessed sexual problems in 9000 women aged 40 to 80 years (inclusive) in 29 countries. The prevalence of inability to reach orgasm ranged from 17.7% (in Northern Europe) to 41.2% (in Southeast Asia). In the PRESIDE survey of over 31,000 women, approximately 10% reported low desire with distress and almost 5% report difficulty reaching orgasm with distress. Anorgasmia is considered to be the persistent or recurrent delay in, or absence of, orgasm following a normal sexual excitement phase, causing marked distress or interpersonal difficulty. When a woman has sexual activity that is not accompanied by good quality orgasmic release, sexual activity may become a chore or a duty rather than a mutually satisfying, intimate experience. This may also lead to secondary loss of sexual interest and/or interpersonal difficulties.

[0804] Testosterone, the primary circulating androgen in women, is a naturally occurring steroid secreted by the ovaries and the adrenal glands. Contrary to the sudden drop in estrogen during menopause, serum levels of androgens fall gradually as women age primarily due to a decrease in the production of adrenal androgen precursors. Testosterone plays a role in regulation of mood, body composition, and bone mineral density, and has central and peripheral effects on sexual function. In the periphery, testosterone is required

for nitric oxide to stimulate vasocongestion for the engorgement of clitoral tissue and vaginal lubrication during sexual arousal. Testosterone stimulates dopamine release in various brain structures implicated in motivation and reward systems, including sexual desire. Testosterone was found to stimulate dopamine release in the medial preoptic area of the anterior hypothalamus under basal conditions and with sexual stimulation in rats.

[0805] The use of androgens to increase women's sexual desire was first reported in 1940 by Loeser. Salmon observed that a number of young married women who formerly considered themselves "frigid" were able to experience "a marked increase in coital gratification, culminating in an orgasm" after testosterone propionate injections, with the effects wearing off within several weeks after the discontinuation of the injections. In the 1980s, the role of androgens in maintaining sexual functioning was studied in oophorectomized women. In this 3-month, prospective open-label study of 44 women, monthly injections of estrogen and testosterone increased rates of sexual desire, sexual arousal, and number of fantasies. Furthermore, rates of intercourse and orgasm were higher in women treated with androgens and estrogen compared to the controls. Over the two past decades, over 80 studies have been conducted in women with hypoactive sexual desire disorder by using exogenous testosterone through the oral, transdermal, sublingual or parenteral route of administration with or without concomitant estrogen therapy, resulting in an increase in sexual desire, orgasm, arousal, frequency of satisfactory sexual activity, pleasure and responsiveness.

[0806] Trimel has developed an intranasal testosterone gel containing 0.24% to 0.72% testosterone with castor oil, oleoyl polyoxylglycerides, and colloidal silicon dioxide as excipients. TBS-2 is administered as a dose applied equally into each nostril. The formulation has many advantageous features including rapid absorption into systemic circulation and rapid clearance, the lack of first-pass metabolism, the avoidance of transference from one person to another, and ease of use. It is a logical next step in a research program dedicated to investigating the role of testosterone, to investigate whether TBS-2, in the absence of other FSDs, has a direct effect on sexual function in general and anorgasmia in particular.

[0807] Two pharmacokinetic (PK)/pharmacodynamic (PD) studies have been performed to evaluate the effect of testosterone on the amygdala reactivity and PD endpoints associated with sexual stimulation. The first PD study (CMO-nr: 2004/144) investigated whether the age-related decline in androgen levels was associated with reduced amygdala activity, and whether exogenous testosterone could restore amygdala activity. The enhanced testosterone levels correlated positively with superior frontal cortex responses and negatively with orbitofrontal cortex responses across individuals, which may reflect testosterone-induced changes in amygdala regulation. These results support the modulatory role of testosterone on emotional cues suggesting testosterone helps to enhance sensation that is necessary to trigger orgasm.

[0808] The second study (TBS-2-2010-01) evaluated the PK of 3 dose levels of TBS-2 and sexual function PD compared to a testosterone patch and placebo. The PK results demonstrated a linear increase in plasma testosterone levels with increasing dose levels. During the first PK series, the mean plasma testosterone concentration and area under the

plasma concentration-time curve (AUC) in the TBS-2 high-dose group reached the same level as the mean concentration and AUC of the testosterone patch at steady state. During the second PK series, the mean plasma testosterone concentration and AUC in the TBS-2 high-dose group reached levels higher than the mean plasma testosterone concentration and AUC in the testosterone patch group, but still within the upper limit of the normal physiological range. Sexual function PD efficacy was explored by assessing the role of testosterone on vaginal pulse amplitude (VPA), subjective arousal questionnaires, and validated computer tasks. Significant differences were observed in VPA response following testosterone administration in women in the anorgasmia cohort. Women who received TBS-2 (see Examples 9 and 10 above) had greater responsiveness in genital response and subjective sexual measurement compared to women receiving the testosterone patch.

[0809] The product under investigation in this study, TBS-2 at 0.24%, 0.48% and 0.72% strengths, is a bioadhesive intranasal testosterone gel. Contrary to the transdermal administration (Intrinsa, a testosterone transdermal patch that has been approved in the European Union for the treatment of hypoactive sexual desire disorder (HSDD) in bilaterally oophorectomized and hysterectomized [surgically induced menopausal] women receiving concomitant estrogen therapy), administration of the bioadhesive TBS-2 through the nasal mucosa allows for rapid absorption into the systemic circulation. The rapid onset and higher peak concentration are hypothesized to be more effective in enhancing sexual desire and orgasm with lower total concentrations of testosterone needed, thus increasing efficacy and decreasing side effects. In addition, TBS-2 may prove effective in alleviating anorgasmia in an "as needed" way, thus avoiding chronic exposure to testosterone.

8. Study Objectives

[0810] A. Primary Objective

[0811] The primary objective of this study was to assess the bioavailability of total testosterone through PK profiles obtained following single administration of an intranasal application of TBS-2 at doses of 600 µg, 1200 µg, and 1800 µg, and multiple administration of 1200 µg TBS-2 given 3 times daily (t.i.d) for the first 2 days and once on the morning of the third day.

[0812] B. Secondary Objectives

[0813] The secondary objectives of this study were:

[0814] To assess the bioavailability of free testosterone, sex hormone-binding globulin

[0815] (SHBG), dihydrotestosterone (DHT), and estradiol through PK profiles obtained following single administration of an intranasal application of TBS-2 at doses of 600 µg, 1200 µg, and 1800 µg, and multiple administration of 1200 µg TBS-2 given for 3 days (t.i.d. for the first 2 days and once on the morning of the third day).

[0816] To evaluate the safety of TBS-2.

9. Investigational Plan

[0817] A. Overall Design and Plan Description

[0818] This was a phase 1, single-center, randomized, open-label parallel-group study in healthy, normal-cycling adult women. Approximately 24 healthy adult women were to be enrolled. Subjects were randomly assigned on a 1:1:1 basis to 1 of 3 treatment groups (Cohort 1, Cohort 2, or Cohort 3)

during Period 1 and were administered a single dose of an intranasal application of TBS-2 at doses of 600 µg, 1200 µg, or 1800 µg (single doses of 300 µg, 600 µg, and 900 µg per nostril). At the end of Period 1, a total of 8 subjects sampled from these 3 cohorts, who were willing and able to continue with the multiple-dose portion of the study were selected to participate in Period 2. During Period 2, subjects were administered 1200 µg TBS-2 (600 µg per nostril) t.i.d. for 2 days and once on the morning of the third day.

[0819] Subjects were screened (Visit 1) for eligibility up to 3 weeks prior to dosing in Period 1, and were admitted to the Clinical Research Unit (CRU) at 0700 hours on the day prior to dosing (Visit 2, Day 1) for baseline testosterone measurement. On Day 2, subjects were administered a single dose of TBS-2 and remained in the CRU for 72 hours post-dose (Day 4) for safety monitoring and PK assessments. Subjects were discharged from the clinic on Day 4, and subjects who did not continue into Period 2 also underwent close-out assessments. Depending on their cycle, subjects selected to participate in Period 2 returned to the CRU approximately 26 to 32 days following the conclusion of Period 1 for Visit 3 (Period 2). During Period 2, subjects were admitted to the CRU at 0700 hours on the day of dosing (Visit 3, Day 1). On Days 1 and 2, subjects were administered TBS-2 at 0800 hours (± 30 minutes), 1600 hours (± 30 minutes), and 2400 hours (± 30 minutes). On Day 3, subjects were administered TBS-2 at 0800 hours. Subjects remained in the CRU for 48 hours following dosing on Day 3 for safety monitoring and PK assessments. Subjects were discharged from the clinic on Day 5.

[0820] During Period 1, blood samples for determination of baseline testosterone (free and total), SHBG, dihydrotestosterone (DHT), and estradiol concentration were collected on Day 1 at 0745 hours and then at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, and 23.5 hours relative to an 0800 hour clock time. Blood samples for determination of testosterone (free and total), SHBG, dihydrotestosterone, and estradiol concentration were collected on Day 2 (15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, and 20 hours post-dose) and Day 3 (24, 32, 40, and 48 hours post-dose) during the confinement period. During Period 2, a blood sample for a baseline serum testosterone concentration was collected at 0745 hours (ie, 15 minutes prior to study drug administration). Blood samples for determination of testosterone (free and total), SHBG, dihydrotestosterone, and estradiol plasma concentration were collected on Day 1 (pre-dose pre-dose [15 minutes prior to dosing] and at 1545 and 2345 hours), Day 2 (1545 and 2345 hours), Day 3 (15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, and 20 hours), and Day 4 (24, 32, 40, and 48 hours) during the confinement period.

[0821] Other assessments performed during the study included the monitoring of adverse events (AEs), clinical laboratory evaluations (chemistry [including hormone profile], hematology, and urinalysis), vital signs assessments (systolic and diastolic blood pressure [BP], heart rate [HR], respiratory rate [RR], and body temperature), and physical examinations. In addition, otorhinolaryngological examination findings, 12-lead electrocardiogram (ECG) readings, medical history, and concomitant medication use were recorded.

[0822] B. Discussion of Study Design and Choice of Control Groups

[0823] This was a phase 1, single-center, randomized, open-label parallel-group study of TBS-2 in 3 cohorts of

subjects (Cohorts 1, 2, and 3) in Period 1 (single-dose) and a multiple-dose cohort in Period 2. Approximately 24 healthy women were to receive a single intranasal dose of 600 µg, 1200 µg, or 1800 µg TBS-2 to evaluate the safety, tolerability, and PK of TBS-2. Subjects were confined in a CRU for 4 days during Period 1 and 5 days during Period 2.

[0824] C. Selection of Study Population

Inclusion Criteria

[0825] Subjects were eligible for study inclusion if they met all of the following inclusion criteria:

[0826] 1. Female subjects between 18 and 40 years of age.

[0827] 2. Subjects with regular menstrual cycles between 26 and 32 days.

[0828] 3. Women of childbearing potential must have agreed to use 1 of the following reliable birth control methods prior to the study, during the study, and up until 1 month after the end of the study:

[0829] a. Surgically sterile (tubal ligation).

[0830] b. Intrauterine device in place for at least 3 months prior to study initiation.

[0831] c. Barrier method (condom with spermicidal agent use by partner).

[0832] d. Abstinence.

[0833] 4. Subjects negative for drugs of abuse, hepatitis B-surface antigen (HbsAg), hepatitis C, human immunodeficiency virus (HIV), and pregnancy (serum β -HCG).

[0834] 5. Subjects with a Body Mass Index (BMI) between 18.5 kg/m² and 35 kg/m² (inclusive).

[0835] 6. Subjects with a normal ear, nose, throat (ENT) examination.

[0836] 7. Subjects with normal thyroid-stimulating hormone (TSH) values.

[0837] 8. Subjects with no clinically significant findings in the physical examination, 12-lead ECG, and vital signs.

[0838] 9. Subjects with normal thyroid function. Physiological prolactin concentration.

[0839] 10. Subjects with no clinical laboratory values outside of the acceptable range, unless the PI decided that they were not clinically significant.

[0840] 11. Subjects who were able to understand and provide written informed consent.

[0841] 12. Subjects who were available for the entire study period and were willing to adhere to protocol requirements, as evidenced by a signed ICF.

Exclusion Criteria

[0842] Subjects were excluded from study participation if they met any of the following exclusion criteria:

[0843] 1. Known history of hypersensitivity to testosterone (eg, Intrinsa patch) and/or related drugs.

[0844] 2. Known history of polycystic ovarian syndrome.

[0845] 3. Known history or presence of cardiac, pulmonary, gastrointestinal, endocrine, musculoskeletal, neurological, psychiatric, hematological, reproductive, liver, or kidney disease, unless judged not clinically significant by the PI or medical designate.

[0846] 4. Presence of or known history of estrogen-responsive tumors such as breast cancer and/or history of any cancer, excluding basal cell carcinoma.

[0847] 5. Known history of frequent clinically significant acne.

[0848] 6. Known history of hirsutism.

- [0849] 7. History of nasal surgery, specifically turbino-plasty, septoplasty, rhinoplasty, "nose job," or sinus surgery.
- [0850] 8. Prior nasal fractures.
- [0851] 9. Active allergies, such as rhinitis, rhinorrhea, and nasal congestion.
- [0852] 10. Mucosal inflammatory disorders, specifically pemphigus and Sjogren's syndrome.
- [0853] 11. Sinus disease, specifically acute sinusitis, chronic sinusitis, or allergic fungal sinusitis.
- [0854] 12. History of nasal disorders (eg, polyposis, recurrent epistaxis [>1 nose bleed per month], abuse of nasal decongestants) or sleep apnea.
- [0855] 13. Use of any form of intranasal medication delivery, specifically nasal corticosteroids and oxymetazoline containing nasal sprays (eg, Dristan 12-Hour Nasal Spray).
- [0856] 14. History of hepatitis B, a positive test for HbsAg, a history of hepatitis C, a positive test for hepatitis C antibody, a history of HIV infection, or demonstration of HIV antibodies.
- [0857] 15. Any history of severe allergic reaction including drugs, food, insect bites, environmental allergens, or any condition known to interfere with the absorption, distribution, metabolism, or excretion of drugs.
- [0858] 16. Any history of drug abuse or alcohol abuse as per the Diagnostic and Statistical
- [0859] Manual of Mental Disorders (fourth edition) criteria within 6 months of study drug administration.
- [0860] 17. Current treatment with any hormonal therapy within previous 12 months, treatment with drugs that interfere with metabolism of testosterone within 30 days of study drug administration and/or any other prescription medications. Difficulty in abstaining from over-the-counter (OTC) medication use for the duration of study.
- [0861] 18. Use of oral, transdermal, and implant contraceptives within 30 days prior to study drug administration or a depot contraceptives injection within 1 year prior to study drug administration.
- [0862] 19. Evidence of pregnancy or lactation.
- [0863] 20. Subjects who were breast feeding or had breast fed within 6 months prior to the screening visit.
- [0864] 21. Administration of another investigational drug within 30 days prior to study drug administration.
- [0865] 22. Blood donation within 56 days prior to study drug administration.
- [0866] 23. Any participation as a plasma donor in a plasmapheresis program within 7 days preceding this study.
- [0867] 24. Intolerance to venipuncture.
- [0868] 25. History of abnormal bleeding tendencies or thrombophlebitis unrelated to venipuncture or intravenous cannulation.
- [0869] 26. History of deep venous thrombosis or coagulation disorders.

Removal of Subjects from Therapy or Assessment

- [0870] Subjects withdrawn from the study after receiving study drug were not replaced, regardless of the reason for withdrawal.
- [0871] A subject may have been prematurely discontinued from the study for any of the following reasons:
 - [0872] Significant noncompliance with the study protocol and procedures.
 - [0873] Intercurrent illness that interfered with the progress of the study.

- [0874] Intolerable AE, including clinically significant abnormal laboratory findings, which in the opinion of the PI could have interfered with the subject's safety.

- [0875] Principal Investigator's decision that the withdrawal from the study was in the best interest of the subject.

[0876] D. Treatments

Treatments Administered

[0877] Subjects were randomly assigned on a 1:1:1 basis to 1 of 3 treatment groups (Cohort 1, Cohort 2, or Cohort 3) during Period 1 and were administered a single dose of an intranasal application of TBS-2 at doses of 600 μ g, 1200 μ g, or 1800 μ g (single doses of 300 μ g, 600 μ g, and 900 μ g per nostril). At the end of Period 1, a total of 8 subjects sampled from these 3 cohorts, who were willing and able to continue with the multiple-dose portion of the study were selected to participate in Period 2. During Period 2, subjects were administered 1200 μ g TBS-2 (600 μ g per nostril) t.i.d. for 2 days and once on the morning of the third day.

[0878] Treatment 1: TBS-2 dispensers prefilled with 0.24% testosterone gel to deliver a single dose of 300 μ g of testosterone per nostril, for a total dose of 600 μ g given at 0800 hours (± 30 minutes) on Day 2 of Period 1 for Cohort 1.

[0879] Treatment 2: TBS-2 dispensers prefilled with 0.48% testosterone gel to deliver a single dose of 600 μ g of testosterone per nostril, for a total dose of 1200 μ g given at 0800 hours (± 30 minutes) on Day 2 of Period 1 for Cohort 2.

[0880] Treatment 3: TBS-2 dispensers prefilled with 0.72% testosterone gel to deliver a single dose of 900 μ g of testosterone per nostril, for a total dose of 1800 μ g given at 0800 hours (± 30 minutes) on Day 2 of Period 1 for Cohort 3.

[0881] Treatment 4: TBS-2 dispensers prefilled with 0.48% testosterone gel to deliver a single dose of 600 μ g of testosterone per nostril, for a total dose of 1200 μ g given t.i.d. daily at 0800 hours (± 30 minutes), 1600 hours (± 30 minutes), and 2400 hours (± 30 minutes) on Days 1 and 2 of Period 2, and once in the morning at 0800 hours (± 30 minutes) on Day 3 of Period 2 (Multidose group).

[0882] Subjects were instructed on the proper procedure for applying the TBS-2 gel intranasally by using the prefilled dispensers. Study drug was self-administered at 0800 hours on Day 2 of Period 1 and on Days 1, 2, and 3 of Period 2. Self-administration of TBS-2 was monitored by study personnel. Subjects were instructed to not blow their nose or sniff immediately, and for the first hour following study drug administration.

Identity of Investigational Products

[0883] Active study drug was supplied in prefilled dispensers containing 0.24%, 0.48%, or 0.72% TBS-2.

[0884] The TBS-2 gels were packaged in prefilled dispensers. A multidose dispenser was used for gel deposition into the nasal cavity. The dispenser was a finger actuated dispensing system designed to dispense 125 μ L of TBS-2 gel per actuation from a non-pressurized container into the nasal cavity. The key components of the multiple-dose dispenser included a barrel, base, pump, and actuator, which were composed of polypropylene and a piston, which was composed of polyethylene. See FIG. 39 and CMC Section below.

[0885] All study drug boxes and dispensers were labeled and supplied according to applicable regulatory requirements. Qualified licensed study personnel prepared the unit

doses for the study as per the randomization schedule. Study drug was provided to the subjects in appropriate unit-dose foil pouches, clearly labeled as to the amount of drug being given. [0886] Study drug was stored in a secure location at a controlled room temperature of 15° C. to 25° C. (59° F. to 77° F.). The storage location was a locked room with limited access, available to appropriate study personnel only.

[0887] The PI, or an approved representative, (eg, co-investigator), ensured that all study drug was stored in a secured area, under recommended storage conditions, and in accordance with applicable regulatory requirements.

[0888] Upon the completion or termination of the study, and upon written authorization from Trimel, all unused and/or partially used study drug was returned or destroyed at the investigational site, as specified by Trimel.

Method of Assigning Subjects to Treatment Groups

[0889] A randomization schedule was prepared by Premier Research and was provided to the PI at the CRU prior to the start of the study.

[0890] Subjects who met the enrollment criteria were randomly assigned on a 1:1:1 basis to 1 of 3 treatment groups.

Selection of Doses in the Study

[0891] The doses of 600 µg, 1200 µg, and 1800 µg were selected for this study based on clinical pharmacology data and are appropriate for a phase 1 study. The animal toxicology studies completed to date do not suggest any unusual or unexpected toxicities related to TBS-2 exposure. Subjects were not expected to experience side effects with TBS-2.

Selection and Timing of Dose for Each Subject

[0892] Study drug was administered on Day 2 of Period 1 (single-dose). During Period 2 (multi-dose), study drug was administered t.i.d. on Days 1 and 2 and on the morning of Day 3. Subjects were confined in a CRU for 4 days during Period 1 and 5 days during Period 2.

Blinding

[0893] This was an open-label study; subjects were not blinded to treatment assignments.

Prior and Concomitant Therapy

[0894] The use of any prescription or nonprescription medication or treatment with drugs that interfere with the metabolism of testosterone was prohibited within 30 days of study drug administration and until the final study visit. The

use of any form of intranasal medication delivery, specifically nasal corticosteroids and oxymetazoline-containing nasal sprays (eg, Dristan 12-Hour Nasal Spray) was prohibited until the final study visit. Current treatment with any hormonal therapy was prohibited 12 months prior to study drug administration and until the final study visit. Also prohibited was the use of oral, transdermal, or implant contraceptives within 30 days prior to drug administration or a depot contraceptive injection within 1 year prior to study drug administration. In addition, the administration of another investigational drug was prohibited within 30 days prior to study drug administration. The use of illegal drugs was not permitted while the subjects were enrolled in this study.

[0895] Throughout the study, the PI could prescribe any concomitant therapy deemed necessary to provide adequate supportive care. The PI was to notify the sponsor of any subject using medications within 30 days prior to Day 1 or if concomitant medications were required during the study. The decision to allow the subject to be enrolled into the study or take medications during the study was made jointly by the sponsor and PI and was based on their opinion that the use of the medication was unlikely to compromise the safety of the subject or the interpretation of the study data. All medications taken within 30 days prior to dosing and all concomitant therapy were recorded on the appropriate section of the CRF.

Treatment Compliance

[0896] Subjects who received doses of study drug were monitored by study personnel for 1 hour post-dose to assure conformity to the TBS-2 instructions. Subjects were confined in a CRU for 4 days during Period 1 and 5 days during Period 2 and were closely monitored. A drug dispensing log was maintained. Pharmacokinetic results were available to confirm compliance.

[0897] Study drug was administered on Day 2 of Period 1 (single-dose). During Period 2 (multi-dose), study drug was administered t.i.d. on Days 1 and 2 and on the morning of Day 3. Subjects were confined in a CRU for 4 days during Period 1 and 5 days during Period 2.

[0898] E. Pharmacokinetic and Safety Variables

Pharmacokinetic and Safety Measurements Assessed and Flow Chart

[0899] The Study Schedule is presented in Table 9-1.

TABLE 9-1

PROCEDURE	Study Schedule										
	Screening	Period 1				Post-Study	Period 2				Post-Study
	Visit 1 Day -21	Visit 2 (Cohorts 1, 2, and 3)					Visit 3 ^a				Visit 3 ^a
	to -1	1	2	3	4	4	1	2	3	4	5
Informed Consent	X										
Inclusion/Exclusion	X										
Confinement			X	X	X		X	X	X	X	
Vital Signs ^b	X	X	X	X	X	X	X	X	X	X	X
ECG	X										
Urine Drug Screen	X	X					X				
Breath Alcohol Testing	X	X					X				

TABLE 9-1-continued

Study Schedule											
	Screening Visit 1 Day -21	Period 1 Visit 2 (Cohorts 1, 2, and 3)			Post- Study	Period 2 Visit 3 ^a				Post- Study	
PROCEDURE	to -1	1	2	3	4	4	1	2	3	4	5
ENT (otorhinolaryngological) Nasal Endoscopic Examination	X										
Basic ENT (Otorhinolaryngological) Examination		X ^c				X ^c	X				X ^c
Medical History	X										
Physical Examination	X					X					X
Serum Pregnancy Test	X	X				X	X				X
Height/Weight	X										
Clinical Laboratory Tests ^d	X					X					X
Serology (HIV, HbsAg, hepatitis C antibody)	X										
Hormone Profile ^e	X					X					X
Dosing			X ^f				X ^g	X ^g	X ^h		
PK Sampling		X ⁱ	X ^j	X ^j	X ^j		X ^k	X ^l	X ^m	X ^m	
Adverse Events			X	X	X	X	X	X	X		X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AE = adverse event; CBC = complete blood count; DHT = dihydrotestosterone; ECG = electrocardiogram; ENT = ear, nose, throat; FSH = follicle-stimulating hormone; HbsAg = hepatitis B surface antigen; HIV = human immunodeficiency virus; LH = luteinizing hormone; PK = pharmacokinetic; SHBG = sex hormone-binding globulin; TSH = thyroid-stimulating hormone.

^aA total of 8 subjects who were willing and able to continue on to the multiple dose portion of the study were selected from Cohorts 1, 2, and 3 and comprised the Multi-Dose group.

^bVital signs (heart rate, blood pressure, temperature, and respiratory rate).

^cThe site physician examined subjects and identified any clinically significant changes to the nasal mucosa at follow-up.

^dChemistry, hematology, urinalysis.

^eHormone profile: TSH, total and free tri-iodothyronine, total and free thyroxine, FSH, prolactin, and progesterone.

^fStudy drug administered at 0800 hours (±30 minutes)

^gStudy drug administered at 0800 hours (±30 minutes), 1600 hours (±30 minutes), and 2400 hours (±30 minutes).

^hStudy drug administered at 0800 hours (±30 minutes).

ⁱBlood samples for the 24-hour baseline profile (total and free testosterone, SHBG, DHT, and estradiol) were collected at 0745 hours and then at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, and 23.5 hours relative to an 0800 hour clock time.

^jBlood samples for the 48-hour PK profile (total and free testosterone, SHBG, DHT, and estradiol) were collected at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 hours.

^kA baseline serum testosterone blood sample was collected at 0745 hour (ie, 15 minutes prior to dosing).

^lPK sampling for trough level measurement was done prior to dosing at 0800, 1600, and 2400 hours.

^mThe 48-hour PK profile blood samples were collected pre-dose at 0745 hours and at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 hours.

Appropriateness of Measurements

[0900] The measurements included in this study were typical for a phase I single- and multiple-dose study.

Pharmacokinetic Drug Concentration Measurements

[0901] Whole blood samples for determination of testosterone (free and total), SHBG, dihydrotestosterone, and estradiol concentration were collected at each time point indicated in Table 9-2.

The date and time of collection were recorded on the CRF to the nearest minute. Whole blood samples were obtained through direct venipuncture; the use of a heparin lock or IV indwelling catheter was allowed for early time point PK sample collections. The volume of plasma samples collected for PK analysis for each subject was approximately 120 mL during Period 1 and 92 mL during Period 2.

TABLE 9-2

Schedule of Pharmacokinetic Sample Collection		
Visit/Day	Treatment	PK Sample Collection
Visit 1/Day 1	None; Baseline measurement	Blood samples for 24-hour baseline profile (total and free testosterone, SHBG, DHT, and estradiol) were collected at 0745 hours and then at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, and 23.5 hours relative to an 0800 hour clock time.
Visit 2/Days 2-4	Single-dose (Cohorts 1, 2, and 3)	Blood samples for the 48-hour PK profile (total and free testosterone, SHBG, DHT, and estradiol) were collected at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 hours.
Visit 3/Day 1	Multiple-dose	A baseline serum blood sample was collected at 0745 hour (ie, 15 minutes prior to dosing).

TABLE 9-2-continued

Schedule of Pharmacokinetic Sample Collection		
Visit/Day	Treatment	PK Sample Collection
Visit 3/Days 1-2	Multiple-dose	PK sampling for trough level measurement was done prior to dosing at 0800, 1600, and 2400 hours.
Visit 3/Days 3-5	Multiple-dose	The 48-hour PK profile blood samples were collected pre-dose at 0745 hours (pre-dose) and at 15, 30, and 45 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 hours.

Abbreviations:

DHT = dihydrotestosterone;

PK = pharmacokinetic;

SHBG—sex hormone-binding globulin;

t.i.d. = three times daily

Note:

During Period 1 dosing occurred on the morning of Day 2. During Period 2, dosing occurred t.i.d. on Days 1 and 2, and once on the morning of Day 3.

Pharmacokinetic Parameter Estimates

[0902] Concentration-time data for 5 analytes [testosterone (Total and Free), SHBG, dihydrotestosterone and estradiol] were determined and PK parameters were calculated from them. Actual sampling time points were recorded and used in calculation of the actual elapsed time from dose to sample for PK calculations. As each administration was made to each of the nostrils, the time of dosing was the time of the first nostril administration. The units of concentration for each of the 5 different analytes may have differed so the final units of concentration reflected the bioanalytical laboratory data reported on the concentrations. Units of PK parameters were derived from the analyte concentration units. Baseline analyte concentrations from the 24-hour pre-dose profile were subtracted from the reported analyte concentrations before calculation of the PK parameters.

[0903] The following PK parameters were calculated following single-dose (Cohorts 1, 2, and 3) for PK characterization. Profile intervals were from the start of the first dosing and continued to the last sample collected on the dosing day. PK parameters were estimated by standard methods used by the WinNonlin program and short descriptions are given below. The determination of the terminal elimination phase was more fully described in the WinNonlin documentation. Note that C_{min} , C_{max} , and t_{max} were taken from the actual measured values but after baseline correction for C_{min} and C_{max} .

Parameter	Description and Calculation
AUC_{0-t}	Area under the plasma concentration time curve from time zero to the last measurable concentration time point, calculated by using the combination of the linear and log trapezoid rules. The linear trapezoidal rule would be used from the time of dose to t_{max} and the log trapezoidal rule would be used following t_{max} .
AUC_{0-8}	Area under the plasma concentration time curve from time zero to 8 hours.
AUC_{0-24}	Area under the plasma concentration time curve from time zero to 24 hours.

-continued

Parameter	Description and Calculation
$AUC_{0-\infty}$	Area under the plasma concentration time curve from time zero to infinity, calculated as $AUC_{0-\infty} = AUC_{0-t} + \frac{C_{last}}{\lambda_z},$ where C_{last} is the last measurable concentration, and λ_z is the terminal elimination rate constant calculated from the log-linear terminal phase.
C_{max}	Maximum concentration observed after dosing.
C_0	Concentration at pre-dose.
C_{24}	Concentration at 24 hours.
t_{max}	Time of observed maximum concentration (C_{max}) relative to the time of dosing.
λ_z	Terminal elimination rate constant.
$t_{1/2}$	Elimination half-life calculated as $\frac{\ln(2)}{\lambda_z}$.

[0904] The following PK parameters were calculated following multiple-dose (Multi-Dose group):

Parameter	Description and Calculation
$AUC_{0-\tau}$	Area under the concentration-time curve from time zero to the dosing interval ($\tau = 8$ hours).
AUC_{0-8}	Area under the plasma concentration time curve from time zero to 8 hours.
AUC_{0-24}	Area under the plasma concentration time curve from time zero to 24 hours.
C_{max}	Maximum concentration observed after dosing.
t_{max}	Time of observed maximum concentration (C_{max}) relative to the time of dosing.
C_{min}	Minimum concentration over a dosing interval during multiple dosing.
C_{pd}	Pre-dose concentration determined immediately before a dose at steady state
C_0	Concentration at pre-dose.
C_{24}	Concentration at 24 hours.
C_{avg}	Average steady-state concentration, calculated as $\frac{AUC_{0-\tau}}{\tau}$, where $\tau = 8$ hours.

-continued

Parameter	Description and Calculation
% PTF	% Peak to Trough Fluctuation $\frac{(C_{max} - C_{min,t}) * 100}{C_{avg}}$
% PTS	% Peak to Trough Swing is the degree of concentration swing at a steady state: $\frac{(C_{max} - C_{min,t}) * 100}{C_{min,t}}$

[0905] Additional exploratory analyses of PK parameters may have been performed as necessary.

Safety Measurements

[0906] Safety measurements included the monitoring of AEs, clinical laboratory evaluations (chemistry [including hormone profile], hematology, and urinalysis), vital signs, and 12-lead ECGs. In addition, physical and otorhinolaryngological examinations were performed and medical history and concomitant medication use were recorded. Safety measurements were performed at the times specified in Table 9-1. The PI followed all clinically-significant abnormal findings after study drug treatment until resolution or return to baseline.

Adverse Events

[0907] An AE is any untoward, undesired, unplanned clinical event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a human participating in a clinical study with a Trimel product, regardless of causal relationship. A pre-existing condition is one that is present prior to study drug administration and is reported as part of the subject's medical history. Pre-existing conditions were reported as an AE only if the frequency, intensity, or character of the pre-existing condition worsened during the course of the study.

[0908] Laboratory abnormalities were not considered AEs unless they were associated with clinical signs or symptoms, or required medical intervention. However, a laboratory abnormality (eg, a clinically significant change detected in clinical chemistry [including hormone profile], hematology, urinalysis) that was independent from the underlying medical condition and that required medical or surgical intervention, or led to study drug interruption or discontinuation, was considered an AE.

[0909] All AEs judged to be clinically significant, including clinically-significant laboratory abnormalities, were followed until resolution or return to baseline.

[0910] F. Severity Rating

[0911] All AEs or SAEs were assessed for severity, by using the following grading scale:

[0912] Mild An event easily tolerated by the subject; transient or mild discomfort (usually <48 hours); no medical intervention/therapy required.

[0913] Moderate An event that may interfere with normal, everyday activities; some assistance may be needed; no or minimal medical intervention/therapy required.

[0914] Severe An event that prevented the subject from performing their normal, everyday activities; some assistance usually required; medical intervention/therapy required, hospitalizations possible.

[0915] When changes in the severity of an AE occurred more frequently than once a day, the maximum severity for the experience that day was noted. If the severity category changed over a number of days, then those changes were recorded separately (with distinct onset dates). The severity of the AE was recorded in the appropriate section of the AE page of the CRF. The evaluation of severity was distinguished from the evaluation of "seriousness." A severe event might not have met the criteria for seriousness and a serious event might have been evaluated as mild.

[0916] G. Causality Rating

[0917] For each reported adverse reaction, the PI made an assessment of the relationship of the event to the study drug by using the following scale:

[0918] Definite There is a plausible temporal relationship with drug administration and withdrawal, and reappears after drug restart.

[0919] Probable There is a plausible temporal relationship with drug administration.

[0920] Possible There is a plausible temporal relationship with drug administration but can reasonably be associated to other factors.

[0921] Unlikely There is no plausible temporal relationship with drug administration.

[0922] Unknown There are no sufficient elements to establish a correlation with drug intake.

[0923] Not Related Cannot be correlated to study drug administration.

[0924] H. Serious Adverse Events

[0925] The PI classified each AE as either serious or not serious. A serious adverse event (SAE) was defined as any AE occurring at any dose that resulted in any of the following outcomes:

[0926] Death^a

[0927] Was life-threatening^b

[0928] Required inpatient hospitalization or prolongation of existing hospitalization

[0929] Resulted in a persistent or significant disability/incapacity

[0930] Resulted in a congenital anomaly/birth defect

[0931] Additionally, important medical events that may not have resulted in death, been life-threatening, or required hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Example: allergic bronchospasm requiring intensive treatment in an emergency room or at home.

[0932] Notes:

[0933] a. Death was an outcome and was NOT the AE. In the event of death, the cause of death was recorded as the AE. The only exception was "sudden death" when the cause was unknown.

[0934] b. Life-threatening AEs included any adverse drug experience, which, in the view of the PI, placed the subject at immediate risk of death from the reaction as it occurred. It did not include a reaction that, had it occurred in a more serious form, might have caused death.

[0935] c. Disability was defined as a substantial disruption in a person's ability to conduct normal life functions.

[0936] All SAEs that resulted in death or were life-threatening, regardless of causal relationship, were reported to Trimel (or designee) within 24 hours of the site's knowledge of the event. A copy of the initial SAE report was to have been

received within 1 business day. All other SAEs or other events reportable to Trimel were forwarded to Trimel (or designee) within 1 business day. If there was any doubt whether the information constituted an SAE, the information was treated as an SAE for the purposes of this study.

Clinical Laboratory Tests

[0937] Clinical laboratory tests were performed according to the schedule provided in Table 9-1.

[0938] Serum chemistry evaluations included sodium, potassium, chloride, glucose, urea, creatinine, calcium, phosphate, uric acid, total bilirubin, albumin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT), creatine kinase (CK), and cholesterol, and hormone profiles.

[0939] Hormone profiles included TSH, total and free triiodothyronine, total and free thyroxine, FSH, prolactin, and progesterone.

[0940] Hematology evaluations included white blood count, hemoglobin, and hematocrit.

[0941] Urinalysis included glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrites, leukocytes and, if necessary, microscopic examination.

[0942] At Screening and on admission to the CRU, a urine specimen was obtained to test for drugs of abuse (marijuana, cocaine, opiates, amphetamines, phencyclidine, benzodiazepines, and barbiturates) and alcohol was tested for by use of an alcohol breath test.

[0943] Testing for HbsAg, antiHCV, and HIV were performed at Screening (Visit 1) only.

[0944] Samples were analyzed by MEDTOX Laboratories, Inc.

Vital Signs

[0945] Vital sign measurements included systolic and diastolic BP and HR, body temperature, and RR. Vital signs assessments were performed at the times specified in Table 9-1.

12-Lead Electrocardiogram

[0946] A standard 12-lead ECG was assessed at Screening (Visit 1) only.

Other Safety Measurements

[0947] Physical examinations were performed at the times specified in Table 9-1.

[0948] An ENT (otorhinolaryngological) nasal endoscopic examination was performed at Screening. Basic ENT (otorhinolaryngological) examinations were performed at the times specified in Table 9-1; the site physician examined subjects and identified any clinically significant changes to the nasal mucosa at follow-up.

[0949] Concomitant medications were monitored throughout the study.

[0950] I. Data Quality Assurance

[0951] This study was monitored by trained experienced personnel in accordance with GCPs. The clinical study monitor reviewed study records to verify adherence to the protocol, accuracy, completeness, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The clinical monitor maintained regular contact

with the site and had access to subject medical records and other study-related records needed to verify the entries on the CRFs.

[0952] Electronic CRFs (eCRFs) were used for this study and included only the subject's initials, date of birth, and an assigned subject number on the eCRFs as identifiers. The PI ensured the availability and reliability of source documents from which the information on the eCRF was derived, and was required to comply with document retention procedures as outlined in the protocol. Case report forms were reviewed for accuracy and signed and dated by the PI.

[0953] J. Statistical Methods and Determination of Sample Size

Statistical and Analytical Plans

[0954] The statistical methods presented in the study protocol were superseded by those described in the statistical analysis plan (SAP). This study evaluated the PK properties as well as the safety and tolerability of TBS-2. Power calculations were not performed. Data were summarized by using descriptive statistics (sample size, mean, median, standard deviation [SD], minimum, and maximum) for each of the safety variables by treatment group and overall. Data from all visits during the study were displayed in the data listings.

[0955] Concentration-time data for 5 analytes (testosterone [total and free], SHBG, dihydrotestosterone, and estradiol) were determined by a validated assay method and PK parameters were calculated. Actual sampling time points were recorded and used in calculation of the actual elapsed time from dose to sample for PK calculations. As each administration was to each of the nostrils, the time of dosing was the time of the first nostril administration. Baseline analyte concentrations from the 24-hour pre-dose profile were subtracted from the time-matched analyte concentrations following dose administration before calculation of the PK parameters.

[0956] The plasma TBS-2 concentration-time data were analyzed by using Phoenix WinNonlin (Pharsight Corporation). The PK parameters (refer to Pharmacokinetic Parameter Estimates) for testosterone (free and total), SHBG, DHT, and estradiol were calculated by standard noncompartmental methods for all subjects as data permitted. Individual PK parameters estimated for each subject's profile in the PK population were displayed in the data listings. Data were summarized by using descriptive statistics (mean, SD, % coefficient of variation [CV], confidence interval (CI), median, minimum, and maximum) and are presented by treatment group.

[0957] Geometric means were included for AUC and C_{max} estimations and were included for some other PK parameters. By using Generalized Linear Model (GLM) procedure in SAS®, an analysis of variance (ANOVA) was performed on natural logarithmic (ln) transformed parameters AUC_{0-r} , $AUC_{0-\infty}$, $AUC_{0-\tau}$, C_{avg} , and C_{max} and on untransformed parameters $t_{1/2}$ and λ_z at the significance level of 0.05. The intrasubject CV was calculated for AUC_{0-r} , $AUC_{0-\infty}$, $AUC_{0-\tau}$, and C_{max} by using the ANOVA residual error.

[0958] Dose linearity following single-dose administration (Period 1) was assessed after normalizing the AUC_{0-r} , $AUC_{0-\infty}$, and C_{max} to the dose administered.

[0959] The following Period 1 comparisons for PK parameters were made:

[0960] Comparison 1: 600 µg 0.24% TBS-2 versus 1200 µg 0.48% TBS-2;

[0961] Comparison 2: 600 µg 0.24% TBS-2 versus 1800 µg 0.72% TBS-2;

[0962] Comparison 3: 1200 µg 0.48% TBS-2 versus 1800 µg 0.72% TBS-2.

Determination of Sample Size

[0963] The sample size for this study was not determined on the basis of statistical hypothesis testing. Based on typical, early-stage PK studies, groups of 8 subjects per cohort were sufficient to provide adequate clinical information to satisfy the objectives of the study.

Interim Analysis

[0964] No formal interim analysis was performed in this study. However, when the concentration data were received from the bioanalytical laboratory following Period 1 and again following Period 2, all PK TLFs were provided to the limit of data available by using scheduled sampling times rather than the actual scheduled sampling times. Multiple-dose data were made available after Period 2. No quality control testing of the preliminary PK analysis or the preliminary tables, listings, and figures were completed for the interim analysis and no testing of the interim PK parameters were completed for the interim analysis.

[0965] K. Changes in the Conduct of the Study or Planned Analyses

[0966] There were changes to the SAP detailing the planned PK analysis of the data after the first draft analysis was conducted. These fell under the category of additional exploratory analyses of PK parameters as provided for in the SAP.

[0967] The first analysis followed the SAP (corrected tau=8 hours) but found that the elimination half-life could not be adequately estimated during the entire 48 hours of the collection period.

[0968] Pharmacokinetic consultants directed the PK analysis and made changes to the PK analysis plans to best present the PK results. In the final analysis a number of additional PK parameters were added to the analysis and additional tests were performed.

[0969] The first round of consultant comments requested these additions to the PK analysis:

[0970] 1. Individual figures of the 3 subjects who received the 1200 µg dose in both periods. Data include plots of the BLQ-corrected raw data from the Bioanalyst for free, total and dihydro-testosterone. Total of 9 figures with 3 for each of the 3 analytes.

[0971] 2. Recalculate the AUC_{0-8} and AUC_{0-24} on the BLQ-corrected data from the Bioanalyst (ConcBLQ). These parameters were compared by ANOVA between Period 1 and Period 2 in a new table. There were only 3 subjects who received the 1200 µg dose in Period 1 (single-dose) and Period 2 (multi-dose). The ANOVA requested was on the 3 subjects who received the 1200 µg dose and also on all 8 subjects' data.

[0972] The second round of consultant comments requested the following. Both consultants reviewed the PK section of the SAP and had the following comments.

[0973] 1. The AUC was requested for either AUC_{0-8} or AUC_{0-24} using baseline corrected for single dose and not baseline corrected for multiple dose. Both were supplied.

[0974] 2. $AUC_{0-\infty}$ was requested to be limited to the 24 hours that was baseline corrected for single dose.

[0975] 3. Concentration at C_{24} was to be reported as a PK parameter for single-dose.

[0976] 4. The error in the SAP was noted where tau was incorrectly given as 12 hours when it was 8 hours.

[0977] 5. Requested C_0 and C_{24} from the multiple-dose to be added to the PK parameters

[0978] 6. Noted that the C_{avg} was to be based on an 8 hour dosing interval (tau).

10. Study Subjects

[0979] A. Disposition of Subjects

[0980] A total of 24 subjects were enrolled in Period 1; 8 subjects each were randomized into Cohort 1, Cohort 2, and Cohort 3. All 24 subjects completed Period 1.

[0981] Following Period 1, a total of 8 subjects continued to Period 2. All 8 subjects completed Period 2.

[0982] B. Protocol Deviations

[0983] The protocol deviations reported during the study were minor and were not expected to affect the outcome of the study. A total of 6 of 24 subjects (25.0%) had protocol deviations of vital signs assessments missed or PK blood draws out of window by 2 or 3 minutes.

11. Pharmacokinetics Evaluation

[0984] A. Data Sets Analyzed

[0985] Single-Dose Population: All subjects who were randomized and received at least 1 dose of TBS-2 during the study; 24 subjects (100%) were included in this population. (Section 14.1, Table 14.1.1a)

[0986] Multi-Dose Population: All subjects in the Single-Dose Population who were selected to continue in study Period 2; 8 subjects (100%) were included in this population. (Section 14.1, Table 14.1.1b)

[0987] B. Demographic and Other Baseline Characteristics

Demographic Data

[0988] Most subjects in Period 1 were white (19 of 24 subjects [79.2%]), followed in percentage by subjects who were black (4 of 24 subjects [16.7%]) and those who were American Indian or Alaskan Native/white (1 of 24 subjects [4.2%]). A similar number of subjects were not Hispanic or Latino (13 of 24 subjects [54.2%]) and Hispanic or Latino (11 of 24 subjects [45.8%]). Mean (SD) age was 29.8 (5.86) years. Mean (SD) height was 161.02 (6.667) cm, weight was 66.36 (11.378) kg, and BMI was 25.61 (4.079) kg/m². Most subjects had a normal BMI (13 of 24 subjects [54.2%]), followed in percentage by subjects who were overweight (7 of 24 subjects [29.2%]) and subjects who were obese (4 of 24 subjects [16.7%]).

[0989] Most subjects in Period 2 were white (5 of 8 subjects [62.5%]), followed in percentage by those who were black (2 of 8 subjects [25.0%]) and those who were American Indian or Alaskan Native/white (1 of 8 subjects [12.5%]). A similar number of subjects were not Hispanic or Latino (5 of 8 subjects [62.5%]) and Hispanic or Latino (3 of 8 subjects [37.5%]). Mean (SD) age was 30.3 (6.48) years. Mean (SD) height was 160.8 (3.99) cm, weight was 61.13 (9.815) kg, and BMI was 23.65 (3.454) kg/m². Most subjects had a normal BMI (6 of 8 subjects [75.0%]), followed in percentage by those who were overweight (2 of 8 subjects [25.0%]).

Baseline Characteristics

Medical History

[0990] Subject medical history reported at Screening is summarized by body system in Table 11.1.

[0991] Currently active non-exclusionary medical history conditions were reported by at least 10% of subjects overall in the following body systems: nervous system (15 of 24 subjects [62.5%]), genitourinary system (7 of 24 subjects [29.2%]), integumentary system (4 of 24 subjects [16.7%]), and allergic conditions (4 of 24 subjects [16.7%]).

included only the 1200 µg testosterone dose. Each dose was profiled for free and total testosterone, dihydrotestosterone, estradiol, and SHBG. There were 8 subjects in each dose group.

[0999] The results are presented first for the concentrations and then for the PK parameters.

[1000] G. Concentration Results

Period 1: Single Dose Profile

[1001] The mean concentration profiles are presented in the following figures for each of the 5 analytes after baseline

TABLE 11-1

Medical History (Single-Dose Population)								
Body System	Cohort 1 (600 µg) (n = 8)		Cohort 2 (1200 µg) (n = 8)		Cohort 3 (1800 µg) (n = 8)		Total (n = 24)	
	Any History	Currently Active	Any History	Currently Active	Any History	Currently Active	Any History	Currently Active
HEENT	1 (12.5%)	0	1 (12.5%)	0	2 (25.0%)	0	4 (16.7%)	0
Respiratory System	0	0	1 (12.5%)	0	3 (37.5%)	0	4 (16.7%)	0
Musculoskeletal System	3 (37.5%)	0	2 (25.0%)	0	2 (25.0%)	1 (12.5%)	7 (29.2%)	1 (4.2%)
Integumentary System (skin, hair, nails)	0	0	2 (25.0%)	2 (25.0%)	2 (25.0%)	2 (25.0%)	4 (16.7%)	4 (16.7%)
Gastrointestinal System	2 (25.0%)	1 (12.5%)	1 (12.5%)	0	1 (12.5%)	1 (12.5%)	4 (16.7%)	2 (8.3%)
Genitourinary System	2 (25.0%)	0	4 (50.0%)	4 (50.0%)	3 (37.5%)	3 (37.5%)	9 (37.5%)	7 (29.2%)
Nervous System	5 (62.5%)	5 (62.5%)	6 (75.0%)	6 (75.0%)	4 (50.0%)	4 (50.0%)	15 (62.5%)	15 (62.5%)
Endocrine System	1 (12.5%)	0	0	0	0	0	1 (4.2%)	0
Lymphatic System	0	0	0	0	0	0	0	0
Immunological System	0	0	0	0	0	0	0	0
Circulatory System	0	0	0	0	0	0	0	0
Psychiatric/ Neurological System	1 (12.5%)	0	0	0	1 (12.5%)	0	2 (8.3%)	0
Allergic Conditions	1 (12.5%)	1 (12.5%)	0	0	4 (50.0%)	3 (37.5%)	5 (20.8%)	4 (16.7%)

HEENT = head, eyes, ears, nose, and throat

[0992] D. Prior and Concomitant Medications

[0993] No concomitant medications were reported during the study.

[0994] Overall, 10 subjects reported prior medications. Most reported prior medications were over-the-counter analgesic or anti-inflammatory medications and multivitamins.

[0995] E. Measurement of Treatment Compliance

[0996] All subjects were observed during dosing at the study site and were compliant with the study drug administration.

[0997] F. Tabulations of Individual Subject Data

Analysis of Pharmacokinetics

[0998] There were 3 doses profiled pharmacokinetically in this study: 3 doses were profiled for the single-dose period and 1 dose was profiled for the multi-dose period. The single-dose profile included doses of 600, 1200, and 1800 µg testosterone in separate cohorts while the multi-dose period

correction. The free testosterone and total testosterone concentration figures present the clearest relationship of concentration to increasing testosterone dose (FIG. 19 and FIG. 20, respectively). The dihydrotestosterone analyte provides a clear distinction between the 1800 µg dose compared with the lower doses which are not clearly differentiated from each other (FIG. 21). The obverse is observed in the estradiol concentrations where it is the lowest dose of 600 µg, which is clearly distinct from the two higher doses, which are not clearly differentiated from each other (FIG. 22). The SHBG concentrations are not clearly distinguished between administered testosterone doses (FIG. 23).

[1002] The free and total testosterone and to a lesser extent the dihydrotestosterone concentrations are more clearly differentiated between the baseline and the active dose (FIG. 24, FIG. 25, and FIG. 26, respectively). The estradiol and SHBG concentrations have post-dose curves that overlap with the baseline concentrations in the same doses (FIG. 27 and FIG. 28, respectively).

Period 2: Multiple Dose Profiles

[1003] The free testosterone and total testosterone mean figures present the clearest dose effect with the highest concentrations at the time of dose and decreasing to a plateau at about 12-16 hours. The concentrations seem to get back to baseline levels after 10 hours post dose. These two plots track so closely that they seem to be superimposable once the scale is adjusted (FIG. 19 and FIG. 20, respectively). The dihydrotestosterone concentrations also indicate a peak at the time of dose but it is not as clear as with the free and total testosterone (FIG. 21). Little difference in estradiol and SHBG concentrations can be seen over the observation period of the multi-dose profile that can be attributed to the absence of a spike in concentration when the dose was administered. (FIG. 32 and FIG. 33, respectively).

[1004] Subject 522-03 had free testosterone concentrations that didn't show a similar decrease as compared to the other 7 subjects 12 hours after the final dose (FIG. 34). The free testosterone concentrations for Subject 522-03 were also the highest concentrations after 12 hours but not as different from the other 7 subjects as were the free testosterone concentrations (FIG. 35). Relationship to dose is increasingly harder to find for the dihydrotestosterone, estradiol, and SHBG in that order with SHBG concentrations essentially constant for each subject but at different concentrations (FIG. 36, FIG. 37, and FIG. 38, respectively).

[1005] H. Pharmacokinetic Parameter Estimates

[1006] Pharmacokinetic parameters were estimated by using Phoenix WinNonlin Version 6.2.

Period 1: Single Dose Pharmacokinetic Parameter Estimates

[1007] All PK parameter estimates were performed on the first 24 hours with the baseline-corrected concentrations. The PK parameters estimated from the single-dose profiles for each individual are presented in Appendix 16.2, Listing 16.2. 5.1.2a and summarized in Section 14.2, Table 14.4.2a. The following table (Table 11-22) presents the data for the single-dose PK parameters but the SD and median have been omitted.

[1008] All 24 subjects had profiles that estimated the λ_z (terminal elimination rate) for free and total testosterone. Only 12 subjects had profiles that estimated the λ_z for dihydrotestosterone, 11 for estradiol, and 9 for SHBG. Without λ_z , the elimination half-life, $t_{1/2}$, and $AUC_{0-\infty}$ could not be calculated.

[1009] Free Testosterone

[1010] For free testosterone, the mean C_{max} closely tracked the administered dose suggesting proportionality to dose. The mean AUC_{0-8} was proportional from the 600 μg dose to the 1200 μg dose (1.94 ratio compared to 2.00 dose ratio) but was somewhat lower for the 1800 μg dose (3.55 ratio compared to 3.00 dose ratio). Other measures of AUC were less clearly related to dose and did not suggest a proportional relationship to dose as strongly as the AUC_{0-8} . This was probably due to the increase in concentration from the dose being most prominent in the first 8 hours as seen in the figures. The λ_z values were similar across doses.

TABLE 11-22

Free Testosterone Summary (Single-Dose Population)												
Dose (μg)		C_{max} (ng/dL)	t_{max} (hr)	AUC_{0-8} (hr*ng/ dL)	AUC_{0-24} (hr*ng/ dL)	$AUC_{0-\infty}$ (hr*ng/ dL)	AUC_{0-8} (hr*ng/ dL)	λ_z (1/hr)	$t_{1/2}$ (hr)	C_{24} ConcBase (ng/dL)	C_{24} ConcBLQ (ng/dL)	C_{24} ConcBC (ng/dL)
600	N	8	8	8	8	8	8	8	8	8	8	8
	Mean	0.56	0.438	1.85	3.104	3.006	4.489	0.101	10.291	0.448	0.481	0.052
	CV	74.2	59.1	61.5	58.1	55.2	63.8	90.5	48.9	91.9	75	107.2
	Min	0.16	0.25	0.94	1.45	1.41	1.47	0.04	2.21	0.18	0.2	0
	Max	1.45	1	4.32	6.18	5.51	8.76	0.31	15.7	1.44	1.3	0.16
	GeoMean	0.442		1.622	2.711	2.652	3.758	0.079	8.784	0.357	0.401	
1200	N	8	8	8	8	8	8	8	8	8	8	8
	Mean	1.274	0.656	3.598	5.265	5.265	6.099	0.104	7.289	0.346	0.423	0.077
	CV	63.8	73.2	60.8	54.8	54.8	49.3	36.8	27.8	39.7	35.9	42.1
	Min	0.32	0.25	0.87	1.28	1.28	1.64	0.07	3.79	0.2	0.24	0.04
	Max	2.51	1.5	8.16	10.61	10.61	11.42	0.18	9.59	0.53	0.63	0.12
	GeoMean	1.017		3.021	4.488	4.488	5.354	0.099	6.995	0.322	0.4	0.071
1800	N	8	8	8	8	8	8	8	8	8	8	8
	Mean	1.708	1.406	6.562	10.958	10.926	12.314	0.123	6.411	0.314	0.436	0.125
	CV	44.2	137.4	36.8	50.1	50.5	51.1	43.3	34.4	24	25.5	79.5
	Min	0.41	0.25	2.36	3.68	3.68	4.04	0.07	2.9	0.19	0.28	0
	Max	2.53	6	9.46	21.22	21.22	23.23	0.24	10.32	0.41	0.62	0.28
	GeoMean	1.5		6.053	9.615	9.561	10.689	0.115	6.049	.0306	0.423	

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; $AUC_{0-\infty}$ = Area under the plasma concentration time curve from time zero to the last measurable concentration time point; AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; C_{24} ConcBase = baseline concentration; C_{24} ConcBLQ = concentration of active dose; C_{24} ConcBC = baseline-corrected concentration; C_{max} = maximum concentration observed after dosing; CV = % coefficient of variation; GeoMean = geometric mean; λ_z = terminal elimination rate constant; $t_{1/2}$ = elimination half-life; t_{max} = time of observed maximum concentration relative to the time of dosing

Note:

The geometric mean is not normally reported for t_{max} .

Note:

The geometric mean cannot be calculated if Min = 0.

[1011] Total Testosterone

[1012] Total testosterone C_{max} parameters increased with increasing dose as did all of the AUC measures. The λ_z measures were similar across doses. (Table 11-23).

TABLE 11-23

Total Testosterone Summary (Single-Dose Population)												
Dose (μg)		C_{max} (ng/dL)	t_{max} (hr)	AUC ₀₋₈ (hr*ng/dL)	AUC ₀₋₂₄ (hr*ng/dL)	AUC _{0-t} (hr*ng/dL)	AUC _{0-∞} (hr*ng/dL)	λ_z (1/hr)	$t_{1/2}$ (hr)	C_{24} ConcBase (ng/dL)	C_{24} ConcBLQ (ng/dL)	C_{24} ConcBC (ng/dL)
600	N	8	8	8	8	8	8	8	8	8	8	8
	Mean	40.625	0.438	135.313	229.095	223.981	327.448	0.108	9.434	30.138	33.138	4.175
	CV	61.1	59.1	31.6	38.3	39	45.1	91.2	48.6	44.7	36.6	98.9
	Min	15.8	0.25	84.58	127.73	127.79	157.01	0.04	2.03	17.1	16	0
	Max	86.7	1	195.66	396.56	396.56	553.97	0.34	16.5	58	50.7	10.4
	GeoMean	34.058		129.311	215.781	210.961	296.981	0.086	8.106	27.873	31.141	
1200	N	8	8	8	8	8	8	8	8	8	8	8
	Mean	71.063	0.688	225.758	328.002	328.002	372.176	0.118	6.472	23.538	28.013	4.475
	CV	48.6	66.6	41.9	35.8	35.8	27.5	39.5	28.5	43.3	35.2	67.3
	Min	21.9	0.25	65.78	102.59	102.59	161.89	0.07	3.09	13.3	17.2	1.4
	Max	133	1.5	377.85	448.61	448.61	487.25	0.22	9.34	41.1	44.6	11.4
	GeoMean	62.88		203.648	301.821	301.821	355.747	0.112	6.199	21.791	26.582	3.809
1800	N	8	8	8	8	8	8	8	8	8	8	8
	Mean	130.863	1.031	515.848	837.083	834.391	947.786	0.133	6.143	24.35	34.55	10.625
	CV	44.3	118.4	44.6	49.3	49.7	54.5	52.1	34.7	28.8	32.1	100.1
	Min	27.2	0.25	169.05	273.14	273.14	315.07	0.09	2.57	17	25	0
	Max	194	4	899.74	1593.93	1593.93	1989.73	0.27	8.07	38.8	59.8	34
	GeoMean	113.912		464.72	742.547	739.181	830.493	0.121	5.716	23.552	33.314	

AUC₀₋₈ = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC₀₋₂₄ = Area under the plasma concentration time curve from time zero to 24 hours after dosing; AUC_{0-t} = Area under the plasma concentration time curve from time zero to the last measurable concentration time point; AUC_{0-∞} = Area under the plasma concentration time curve from time zero to infinity; C_{24} ConcBase = baseline concentration; C_{24} ConcBLQ = concentration of active dose; C_{24} ConcBC = baseline-corrected concentration; C_{max} = maximum concentration observed after dosing; CV = % coefficient of variation; GeoMean = geometric mean; λ_z = terminal elimination rate constant; $t_{1/2}$ = elimination half-life; t_{max} = time of observed maximum concentration relative to the time of dosing

Note:

The geometric mean is not normally reported for t_{max} .

Note:

The geometric mean cannot be calculated if Min = 0.

[1013] Dihydrotestosterone

[1014] Dihydrotestosterone PK parameters were missing a number of parameter values and some entire profiles were missing after baseline correction; only 18 of the 24 profiles were present and λ_z could be estimated in only 12 of the 24

profiles. Mean C_{max} , AUC₀₋₈, and AUC_{0-∞} values decreased from the 600 μg dose to the 1200 μg dose but then increased for the 1800 μg dose. The other AUC measures demonstrated increasing exposure with increasing dose. The λ_z values were roughly similar for each dose. (Table 11-24)

TABLE 11-24

Dihydrotestosterone Summary (Single-Dose Population)												
Dose (μg)		C_{max} (ng/dL)	t_{max} (hr)	AUC ₀₋₈ (hr*ng/dL)	AUC ₀₋₂₄ (hr*ng/dL)	AUC _{0-t} (hr*ng/dL)	AUC _{0-∞} (hr*ng/dL)	λ_z (1/hr)	$t_{1/2}$ (hr)	C_{24} ConcBase (ng/dL)	C_{24} ConcBLQ (ng/dL)	C_{24} ConcBC (ng/dL)
600	N	6	6	5	5	6	4	4	4	5	5	5
	Mean	7.078	1.75	26.902	39.038	31.844	83.96	0.153	16.229	14.274	14.302	0.828
	CV	70.1	122.6	74.8	61.7	74.9	98.6	127.7	105.6	44.2	31.4	151.3
	Min	2.66	0.25	9.44	11.51	5.61	13.85	0.02	1.58	8.23	8.61	0
	Max	16.27	6	59.82	71.37	70.18	202.98	0.44	39.89	22.1	19	2.97
	GeoMean	5.888		21.859	32.493	23.515	55.582	0.077	9.009	13.144	13.697	
1200	N	6	6	6	6	6	3	3	3	6	6	6
	Mean	6.23	5.083	23.909	45.617	43.261	72.971	0.057	12.43	12.048	12.203	0.867
	CV	43.6	182.4	48.4	52.4	49.1	22.2	19.6	20.6	54.9	39.7	116
	Min	2.27	1	8.32	16.9	16.9	54.27	0.05	10.22	8.03	8.32	0
	Max	9.62	24	37.33	79.02	69.98	82.43	0.07	15.24	25.3	21.8	2.27
	GeoMean	5.646		20.858	39.952	38.457	71.647	0.057	12.259	11.004	11.589	
1800	N	6	6	6	6	6	5	5	5	6	6	6
	Mean	11.392	2.792	52.921	98.838	98.078	158.68	0.1	11.855	11.865	13.615	1.918
	CV	59.3	101.1	68.5	63.4	64.2	73.4	71.6	78	40	37.6	124.6
	Min	3.93	0.75	19.3	41.71	41.71	70.03	0.03	3.7	5.55	8.06	0

TABLE 11-24-continued

Dihydrotestosterone Summary (Single-Dose Population)											
Dose (μg)	C_{max} (ng/dL)	t_{max} (hr)	AUC_{0-8} (hr*ng/dL)	AUC_{0-24} (hr*ng/dL)	AUC_{0-t} (hr*ng/dL)	$AUC_{0-\infty}$ (hr*ng/dL)	λ_z (1/hr)	$t_{1/2}$ (hr)	C_{24} ConcBase (ng/dL)	C_{24} ConcBLQ (ng/dL)	C_{24} ConcBC (ng/dL)
Max	23.4	8	117.76	217.97	217.97	327.95	0.19	23.31	20	21.4	6.4
GeoMean	9.82		43.767	86.043	85.18	128.512	0.077	8.971	11.068	12.834	

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; AUC_{0-t} = Area under the plasma concentration time curve from time zero to the last measurable concentration time point; $AUC_{0-\infty}$ = Area under the plasma concentration time curve from time zero to infinity; C_{24} ConcBase = baseline concentration; C_{24} ConcBLQ = concentration of active dose; C_{24} ConcBC = baseline-corrected concentration; C_{max} = maximum concentration observed after dosing; CV = % coefficient of variation; GeoMean = geometric mean; λ_z = terminal elimination rate constant; $t_{1/2}$ = elimination half-life; t_{max} = time of observed maximum concentration relative to the time of dosing

Note:

The geometric mean is not normally reported for t_{max} .

Note:

The geometric mean cannot be calculated if Min = 0.

[1015] Estradiol

[1016] Estradiol PK parameters for C_{max} and AUC measures were similar for the 1200 μg and 1800 μg doses, but consistently less than the values from the 600 μg dose. For example the mean C_{max} ratios were 0.61 and 0.64 for 600 μg

dose compared to the 1200 μg and 1800 μg doses, respectively. Similar ratios for AUC_{0-8} were 0.31 and 0.47, respectively, where ratios of 2.00 and 3.00 would indicate proportionality. Also note that the 600 μg dose had an N=8 while both the 1200 μg and 1800 μg doses had an N=7. The λ_z values were similar. (Table 11-25)

TABLE 11-25

Estradiol Summary (Single-Dose Population)												
Dose (μg)		C_{max} (pg/mL)	t_{max} (hr)	AUC_{0-8} (hr*pg/ mL)	AUC_{0-24} (hr*pg/ mL)	AUC_{0-t} (hr*pg/ mL)	$AUC_{0-\infty}$ (hr*pg/ mL)	λ_z (1/hr)	$t_{1/2}$ (hr)	C_{24} ConcBase (pg/mL)	C_{24} ConcBLQ (pg/mL)	C_{24} ConcBC (pg/mL)
600	N	8	8	8	8	8	5	5	5	8	8	8
	Mean	23.275	10.816	112.508	236.334	230.951	718.407	0.063	30.458	50.1	47.438	6.863
	CV	128.4	90.3	159	120.4	118.7	69	106.8	119.5	65.4	41.4	81.1
	Min	3.4	0.25	0	10	10	194.98	0.01	3.88	22.9	27.9	0
	Max	92.8	24	528.41	723	679.81	1351.44	0.18	93.63	122	90.6	18
	GeoMean	13.468			106.562	105.763	553.278	0.039	17.818	43.319	44.477	
1200	N	7	7	7	7	7	2	2	2	8	8	8
	Mean	14.186	9.854	34.928	134.419	133.848	383.708	0.067	21.96	40.288	42.6	7.1
	CV	33.9	100.7	110.2	81.3	82.2	51.7	102.7	102.7	40.9	33.1	106.4
	Min	7.8	1	0	9.3	7.3	243.46	0.02	6.02	22.5	26.6	0
	Max	22.2	24	101.1	322.73	322.73	523.95	0.12	37.9	75	70.2	19.9
	GeoMean	13.474			80.078	75.97	357.16	0.046	15.104	37.815	40.76	
1800	N	7	7	7	7	7	4	4	4	8	8	8
	Mean	14.843	7.176	52.593	134.952	132.894	323.544	0.166	7.886	42.55	34.713	4.038
	CV	92.3	143.1	177.8	192.4	197.9	166.1	109.4	63.3	38.3	65	213
	Min	5.4	0.25	1.88	9.74	8.11	22.4	0.06	1.59	23.3	16.5	0
	Max	44.5	24	262.18	722.27	727.84	1128.44	0.44	12.24	65.4	88.2	24.1
	GeoMean	11.47		18.811	48.85	43.97	105.227	0.114	6.103	39.849	30.552	

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; AUC_{0-t} = Area under the plasma concentration time curve from time zero to the last measurable concentration time point; $AUC_{0-\infty}$ = Area under the plasma concentration time curve from time zero to infinity; C_{24} ConcBase = baseline concentration; C_{24} ConcBLQ = concentration of active dose; C_{24} ConcBC = baseline-corrected concentration; C_{max} = maximum concentration observed after dosing; CV = % coefficient of variation; GeoMean = geometric mean; λ_z = terminal elimination rate constant; $t_{1/2}$ = elimination half-life; t_{max} = time of observed maximum concentration relative to the time of dosing

Note:

The geometric mean is not normally reported for t_{max} .

Note:

The geometric mean cannot be calculated if Min = 0.

[1017] SHBG

[1018] The SHBG C_{max} and AUC_{0-8} , AUC_{0-24} , and AUC_{0-t} values were similar for the 600 μg and the 1200 μg doses. The λ_z was not characterized for the majority of these subjects. (Table 11-26)

TABLE 11-26

SHBG Summary (Single-Dose Population)												
Dose (μg)		C _{max} (nmol/L)	t _{max} (hr)	AUC ₀₋₈ (hr*nmol/ L)	AUC ₀₋₂₄ (hr*nmol/ L)	AUC _{0-τ} (hr*nmol/ L)	AUC _{0-∞} (hr*nmol/ L)	λ _z (1/hr)	t _{1/2} (hr)	C ₂₄ ConcBase (nmol/L)	C ₂₄ ConcBLQ (nmol/L)	C ₂₄ ConcBC (nmol/L)
600	N	8	8	8	8	8	4	4	4	8	8	8
	Mean	7.138	4.25	22.987	44.1	40.805	195.593	0.126	20.766	61.413	61.25	1.275
	CV	53.9	84.3	66.7	63.1	72.5	121.3	129.1	93	36.3	37.9	258.4
	Min	2.6	0.25	8.74	13.63	8.02	35.66	0.02	1.92	18.7	17.6	0
	Max	14.3	8	48.46	92.06	92.06	548.32	0.36	40.79	97.2	93.7	9.4
1200	GeoMean	6.284		19.12	37.116	31.916	117.278	0.06	11.53	56.534	55.893	
	N	8	8	8	8	8	2	2	2	8	8	8
	Mean	7.525	5.406	23.798	45.416	40.309	99.885	0.323	4.869	51.738	49.663	0.713
	CV	52.2	91.5	109	81.4	97.7	16.6	105.7	105.7	45.9	43.5	282.8
	Min	1.2	0.25	0.2	7.1	5.29	88.18	0.08	1.23	17.4	17.2	0
1800	Max	11.8	16	80.45	111.62	111.42	111.59	0.56	8.51	86.7	77	5.7
	GeoMean	6.155		9.296	32.213	24.806	99.197	0.214	3.235	46.256	44.723	
	N	8	7	7	6	8	3	3	3	8	8	8
	Mean	0.888	9.013	13.161	34.62	32.988	205.537	0.053	29.135	63.388	64.513	2.25
	CV	101	114.8	76.4	82.5	89.6	65.7	117.5	71.6	52.8	47.1	112.9
	Min	0	0.25	0.54	7.33	0	63.1	0.02	5.54	27.9	31.1	0
	Max	29.3	24.07	26.82	71.26	71.26	331.45	0.13	45.09	116	109	7.3
	GeoMean			8.128	23.258		166.844	0.033	20.941	56.059	58.42	

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; $AUC_{0-\tau}$ = Area under the plasma concentration time curve from time zero to the last measurable concentration time point; $AUC_{0-\infty}$ = Area under the plasma concentration time curve from time zero to infinity; C_{24} ConcBase = baseline concentration; C_{24} ConcBLQ = concentration of active dose; C_{24} ConcBC = baseline-corrected concentration; C_{max} = maximum concentration observed after dosing; CV = % coefficient of variation; GeoMean = geometric mean; λ_z = terminal elimination rate constant; $t_{1/2}$ = elimination half-life; t_{max} = time of observed maximum concentration relative to the time of dosing

Note:

The geometric mean is not normally reported for t_{max} .

Note:

The geometric mean cannot be calculated if Min = 0.

I. Period 2: Multiple Dose Pharmacokinetic Parameter Estimates

[1019] The PK parameters estimated from the multi-dose profiles for each individual are presented in Appendix 16.2, Listing 16.2.5.1.2b and summarized in Section 14.2, Table 14.4.2b. The following tables present the data summarized in Section 14.2, Table 14.4.2b for the multi-dose PK parameters but the SD and median have been omitted. All subjects received 7 doses of 1200 μg testosterone.

[1020] Free Testosterone

[1021] Since the doses of 1200 μg testosterone were used in both the single- and multi-dose periods of this study, it is would seem useful to compare their PK parameters. However, this would not be useful as the single-dose data is based on baseline-corrected data while the multi-dose data is not. However, the baseline concentration data from the single-dose period may be reviewed in comparison to the multi-dose

data. Since the data in Table Table 11-28 are from baseline, the dose is not relevant as all concentrations are prior to dose.

[1022] The minimum and maximum values observed in the multi-dose period are greater than the minimum and maximum values observed in the baseline period for all 3 dose cohorts. This demonstrates that the testosterone multiple dosing are increasing both the minimum and the maximum free testosterone concentrations over the baseline values and provides the expected increase in free testosterone concentrations after multiple dosing. (Table 11-27) and (Table 11-28 Table).

[1023] The AUC_{0-8} and the $AUC_{0-\tau}$ are identical as the dosing interval is 8 hours. The AUC_{0-24} hours spans the last dose period of 8 hours but also includes the preceding 16 hours. The % PTF and % PTS indicate the percent difference between the C_{max} and C_{min} when divided by the C_{avg} or C_{min} , respectively.

TABLE 11-27

Free Testosterone Summary for Multiple Dose Profile (Multi-Dose Population)											
	C_{max} (ng/dL)	t_{max} (hr)	C_0 (ng/dL)	C_{min} (ng/dL)	C_{avg} (ng/dL)	C_{24} (ng/dL)	AUC_{0-8} (hr*ng/dL)	AUC_{0-24} (hr*ng/dL)	$AUC_{0-\tau}$ (hr*ng/dL)	% PTF	% PTS
N	8	8	8	8	8	8	8	8	8	8	8
Mean	1.886	0.378	1.155	0.704	1.006	0.578	8.046	17.259	8.046	140.636	214.058
CV	12.4	49.6	34.2	55.1	40.6	79.8	40.6	55.0	40.6	55.2	55.0
Min	1.65	0.25	0.77	0.40	0.59	0.28	4.69	9.74	4.69	30.60	35.00
Max	2.18	0.75	1.82	1.60	1.83	1.71	14.64	39.66	14.64	295.75	437.88
GeoMean	1.874	0.344	1.098	0.638	0.944	0.492	7.554	15.722	7.554	119.579	176.905

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; $AUC_{0-\tau}$ = Area under the concentration-time curve from time zero to the dosing interval; C_{max} = maximum concentration observed after dosing; C_0 = maximum concentration at baseline; C_{min} = Minimum concentration over a dosing interval during multiple dosing; C_{avg} = Average steady-state concentration; C_{24} = maximum concentration at 24 hours after dosing; CV = % coefficient of variation; GeoMean = geometric mean; % PTF = percent peak to trough fluctuation; % PTS = percent peak to trough swing; t_{max} = time of observed maximum concentration relative to the time of dosing.

TABLE 11-28

Free Testosterone Concentration at Baseline (Single-Dose Population)				
Analyte		Dose Cohort (ug)		
		600	1200	1800
		ConcBase (ng/dL)		
Free Testosterone	N	112	112	112
	Min	0.10	0.13	0.15
	Max	1.44	0.53	0.45

ConcBase = baseline concentration

[1024] Total Testosterone

[1025] The total testosterone PK C_{max} range of 85.80 to 242.00 ng/dL following multiple dosing compares to the maximum testosterone concentration range of 41.10 to 58.00 ng/dL observed at baseline. The C_{min} range of 25.20 to 79.50 ng/dL total testosterone following multiple dosing compares to the 8.00 to 12.70 ng/dL range of minimum value of total testosterone observed at baseline. Both the C_{max} and the C_{min} indicate an effect of the testosterone dose administration; 2- to 4-fold effect on C_{max} and a 3-fold effect on C_{min} . (Table 11-29 and Table 11-30), however, this accumulation disappears within 24 hours after the last administration.

TABLE 11-30

Total Testosterone Concentration at Baseline (Single-Dose Population)				
Analyte		Dose Cohort (ug)		
		600	1200	1800
		ConcBase (ng/dL)		
Total Testosterone	N	112	112	112
	Min	8.00	7.41	12.70
	Max	58.00	41.10	43.90

ConcBase = baseline concentration

[1026] Dihydrotestosterone

[1027] The dihydrotestosterone observations do not indicate much difference from the baseline observations. The maximum dihydrotestosterone C_{max} from the multi-dose period of 30.50 ng/dL is comparable to with the 32.60 C_{max} observed at baseline. The minimum C_{min} from the multi-dose period of 6.32 ng/dL is comparable to the 5.02 ng/dL observed at baseline. No effect of the testosterone administration is seen in this comparison of dihydrotestosterone concentrations to baseline. (Table 11-31 and Table 11-32)

TABLE 11-29

Total Testosterone Summary for Multiple Dose Profile (Multi-Dose Population)											
	C_{max} (ng/dL)	t_{max} (hr)	C_0 (ng/dL)	C_{min} (ng/dL)	C_{avg} (ng/dL)	C_{24} (ng/dL)	AUC_{0-8} (hr*ng/dL)	AUC_{0-24} (hr*ng/dL)	$AUC_{0-\infty}$ (hr*ng/dL)	% PTF	% PTS
N	8	8	8	8	8	8	8	8	8	8	8
Mean	148.038	0.440	85.450	49.088	73.802	38.513	590.419	1224.571	590.419	141.760	216.089
CV	41.5	49.8	32.1	36.4	41.4	47.2	41.4	38.5	41.4	53.8	53.5
Min	85.80	0.25	40.50	25.20	41.12	18.70	329.00	679.18	329.00	48.83	64.47
Max	242.00	0.75	131.00	79.50	141.00	77.30	1127.99	2026.22	1127.99	301.43	447.51
GeoMean	137.555	0.395	81.313	46.136	69.166	35.360	553.325	1147.566	553.325	125.100	187.544

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; $AUC_{0-\infty}$ = Area under the concentration-time curve from time zero to the dosing interval; C_{max} = maximum concentration observed after dosing; C_0 = maximum concentration at baseline; C_{min} = Minimum concentration over a dosing interval during multiple dosing; C_{avg} = Average steady-state concentration; C_{24} = maximum concentration at 24 hours after dosing; CV = % coefficient of variation;

GeoMean = geometric mean; % PTF = percent peak to trough fluctuation; % PTS = percent peak to trough swing; t_{max} = time of observed maximum concentration relative to the time of dosing.

TABLE 11-31

Dihydrotestosterone Summary for Multiple Dose Profile (Multi-Dose Population)											
	C_{max} (ng/dL)	t_{max} (hr)	C_0 (ng/dL)	C_{min} (ng/dL)	C_{avg} (ng/dL)	C_{24} (ng/dL)	AUC_{0-8} (hr*ng/dL)	AUC_{0-24} (hr*ng/dL)	$AUC_{0-\tau}$ (hr*ng/dL)	% PTF	% PTS
N	8	8	8	8	8	8	8	8	8	8	8
Mean	22.300	0.500	20.724	13.465	16.628	14.774	133.024	338.888	133.024	57.240	73.956
CV	35.9	53.5	39.3	44.9	40.0	40.1	40.0	38.6	40.0	44.1	48.7
Min	10.20	0.25	7.39	6.32	7.21	6.33	57.66	145.85	57.66	13.66	14.90
Max	30.50	1.00	31.70	20.80	24.30	24.70	194.43	494.97	194.43	96.25	125.53
GeoMean	20.792	0.442	18.927	12.192	15.274	13.637	122.194	313.198	122.194	50.641	63.444

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; $AUC_{0-\tau}$ = Area under the concentration-time curve from time zero to the dosing interval; C_{max} = maximum concentration observed after dosing; C_0 = maximum concentration at baseline; C_{min} = Minimum concentration over a dosing interval during multiple dosing; C_{avg} = Average steady-state concentration; C_{24} = maximum concentration at 24 hours after dosing; CV = % coefficient of variation; GeoMean = geometric mean; % PTF = percent peak to trough fluctuation; % PTS = percent peak to trough swing; t_{max} = time of observed maximum concentration relative to the time of dosing.

TABLE 11-32

Dihydrotestosterone Concentration at Baseline (Single-Dose Population)					
Analyte		Dose Cohort (ug)			
		600	1200	1800	
		ConcBase (ng/dL)			
Dihydrotestosterone	N	75	78	78	
	Min	5.29	5.04	5.02	
	Max	22.10	25.30	32.60	

ConcBase = baseline concentration

[1028] Estradiol

[1029] The estradiol C_{min} concentration observed over 24 hours during the baseline observation period was 17.00 pg/mL (17.00 to 20.90 pg/mL range of 24 subjects in the single-dose period) which compares to the 12.70 pg/mL (12.70 to 95.20 pg/mL range of 8 subjects) in the multi-dose period. The estradiol C_{max} concentration was 122.00 during the baseline period and 145.00 during the multi-dose period. While there is some difference in these concentrations, they are similar. (Table 11-33 and Table 11-34).

TABLE 11-34

Estradiol Concentration at Baseline (Single-Dose Population)				
Analyte		Dose Cohort (ug)		
		600	1200	1800
		ConcBase (pg/mL)		
Estradiol	N	112	112	112
	Min	20.90	17.00	17.70
	Max	122.00	119.00	97.40

ConcBase = baseline concentration

[1030] SHBG

[1031] The SHBG C_{min} from the baseline observation period was 15.30 nmol/L while the C_{min} from the multi-dose period was 19.90 nmol/L. The SHBG C_{max} was 137.00 nmol/L while the C_{max} from the multi-dose period was 100.00 nmol/L. No effect from the testosterone dosing on SHBG concentrations is obvious. (Table 11-35 and Table 11-36).

TABLE 11-33

Estradiol Summary for Multiple Dose Profile (Multi-Dose Population)											
	C_{max} (pg/mL)	t_{max} (hr)	C_0 (pg/mL)	C_{min} (pg/mL)	C_{avg} (pg/mL)	C_{24} (pg/mL)	AUC_{0-8} (hr*pg/mL)	AUC_{0-24} (hr*pg/mL)	$AUC_{0-\tau}$ (hr*pg/mL)	% PTF	% PTS
N	8	8	8	8	8	8	8	8	8	8	8
Mean	70.800	3.231	54.113	48.875	58.019	64.113	464.148	1759.342	464.148	35.465	42.164
CV	54.9	79.7	44.3	50.6	54.0	44.2	54.0	41.3	54.0	44.8	46.9
Min	16.60	0.27	16.30	12.70	14.96	18.60	119.67	503.91	119.67	20.16	22.22
Max	145.00	8.00	84.20	95.20	120.64	104.00	965.13	2895.80	965.13	66.76	79.82
GeoMean	60.034	2.024	48.062	42.569	50.033	56.820	400.264	1587.754	400.264	32.768	38.514

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing;
 AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing;
 $AUC_{0-\tau}$ = Area under the concentration-time curve from time zero to the dosing interval;
 C_{max} = maximum concentration observed after dosing;
 C_0 = maximum concentration at baseline;
 C_{min} = Minimum concentration over a dosing interval during multiple dosing;
 C_{avg} = Average steady-state concentration;
 C_{24} = maximum concentration at 24 hours after dosing;
CV = % coefficient of variation;
GeoMean = geometric mean;
% PTF = percent peak to trough fluctuation;
% PTS = percent peak to trough swing;
 t_{max} = time of observed maximum concentration relative to the time of dosing.

TABLE 11-35

SHBG Summary for Multiple Dose Profile (Multi-Dose Population)											
	C _{max} (nmol/L)	t _{max} (hr)	C ₀	C _{min} (nmol/L)	C _{avg}	C ₂₄	AUC ₀₋₈	AUC ₀₋₂₄ (hr*nmol/L)	AUC _{0-∞}	% PTF	% PTS
N	8	8	8	8	8	8	8	8	8	8	8
Mean	62.725	1.998	59.700	55.300	58.548	56.275	468.387	1375.463	468.387	13.778	15.114
CV	46.2	136.0	46.2	47.5	45.9	45.5	45.9	45.1	45.9	56.6	64.6
Min	27.40	0.25	25.40	19.90	23.91	25.10	191.29	591.69	191.29	8.10	8.48
Max	100.00	7.98	93.90	87.90	92.65	87.10	741.16	2125.93	741.16	31.37	37.69
GeoMean	56.270	0.964	53.530	49.024	52.530	50.636	420.236	1239.069	420.236	12.392	13.279

AUC_{0-8} = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC_{0-24} = Area under the plasma concentration time curve from time zero to 24 hours after dosing; $AUC_{0-\infty}$ = Area under the concentration-time curve from time zero to the dosing interval; C_{max} = maximum concentration observed after dosing; C_0 = maximum concentration at baseline; C_{min} = Minimum concentration over a dosing interval during multiple dosing; C_{avg} = Average steady-state concentration; C_{24} = maximum concentration at 24 hours after dosing; CV = % coefficient of variation; GeoMean = geometric mean; % PTF = percent peak to trough fluctuation; % PTS = percent peak to trough swing; t_{max} = time of observed maximum concentration relative to the time of dosing.

TABLE 11-36

SHBG Concentration at Baseline (Single-Dose Population)				
		Dose Cohort (ug)		
		600	1200	1800
Analyte		ConcBase (nmol/L)		
SHBG	N	112	112	112
	Min	15.30	16.00	25.20
	Max	102.00	86.70	137.00

ConcBase = baseline concentration

[1032] J. Pharmacokinetic Parameter Statistical Testing

Dose Proportionality Analysis on Single Dose

[1033] Section 14.2, Table 14.4.3 presents the analysis of dose proportionality. Table 11-37 is abstracted from this table to present the linear regression analysis of the log-transformed PK parameters.

[1034] Dose proportionality occurs when increases in the administered dose are accompanied by proportional increases in a measure of exposure (PK parameter). The linear regression model is fitted to each of the interested PK parameters by using dose (600, 1200, and 1800 μ g) as the predictor variable:

$$PK = \text{Slope} * \text{Dose} + \text{Intercept}.$$

[1035] A dose proportionality hypothesis cannot be rejected if the intercept's 95% confidence limit includes 0.

[1036] Dose proportionality was not rejected for only AUC_{0-24} , AUC_{0-8} , and AUC_{0-t} for free testosterone.

TABLE 11-37

Dose Proportionality Analysis (Single-Dose Population)							
Analyte	Log Transformed		Standard Error	95% Confidence Limit for Estimate	N/	R-Square	
	PK Parameter	Regression Estimate		Lower Limit Upper Limit			
Free Testosterone	AUC_{0-24}	Intercept	0.3211	0.3154	-0.3331	0.9753	24
		Slope	0.0011	0.0002	0.0006	0.0016	0.4607
	AUC_{0-8}	Intercept	-0.1868	0.2951	-0.7988	0.4252	24
		Slope	0.0011	0.0002	0.0006	0.0016	0.5136
	$AUC_{0-\infty}$	Intercept	0.7450	0.3266	0.0676	1.4224	24
		Slope	0.0009	0.0003	0.0003	0.0014	0.352
	AUC_{0-t}	Intercept	0.2956	0.3134	-0.3544	0.9456	24
		Slope	0.0011	0.0002	0.0006	0.0016	0.4703
	C_{max}	Intercept	-1.3533	0.3811	-2.1436	-0.5630	24
		Slope	0.0010	0.0003	0.0004	0.0016	0.3529
Total Testosterone	AUC_{0-24}	Intercept	4.6622	0.2607	4.1216	5.2029	24
		Slope	0.0010	0.0002	0.0006	0.0014	0.5437
	AUC_{0-8}	Intercept	4.1608	0.2525	3.6371	4.6845	24
		Slope	0.0011	0.0002	0.0007	0.0015	0.5765
	$AUC_{0-\infty}$	Intercept	5.0683	0.2653	4.5181	5.6185	24
		Slope	0.0009	0.0002	0.0004	0.0013	0.4435
	AUC_{0-t}	Intercept	4.6351	0.2597	4.0965	5.1738	24
		Slope	0.0010	0.0002	0.0006	0.0015	0.5528
	C_{max}	Intercept	2.9275	0.3285	2.2463	3.6088	24
		Slope	0.0010	0.0003	0.0005	0.0015	0.4174
Dihydrotestosterone	AUC_{0-24}	Intercept	2.8766	0.4092	2.0043	3.7488	17
		Slope	0.0008	0.0003	0.0002	0.0015	0.3234
	AUC_{0-8}	Intercept	2.5722	0.4515	1.6098	3.5347	17
		Slope	0.0006	0.0003	-0.0001	0.0013	0.1713
	$AUC_{0-\infty}$	Intercept	3.5478	0.5806	2.2542	4.8415	12
		Slope	0.0007	0.0004	-0.0003	0.0017	0.2131
	AUC_{0-t}	Intercept	2.4635	0.4276	1.5571	3.3699	18
		Slope	0.0011	0.0003	0.0004	0.0018	0.3979

TABLE 11-37-continued

Dose Proportionality Analysis (Single-Dose Population)							
Analyte	Log Transformed PK Parameter	Regression	Estimate	Standard Error	95% Confidence Limit for Estimate		N/ R-Square
					Lower Limit	Upper Limit	
	C_{max}	Intercept	1.4180	0.3725	0.6283	2.2076	18
		Slope	0.0004	0.0003	-0.0002	0.0010	0.1209

AUC₀₋₈ = Area under the plasma concentration time curve from time zero to 8 hours after dosing; AUC₀₋₂₄ = Area under the plasma concentration time curve from time zero to 24 hours after dosing; AUC_{0-∞} = Area under the plasma concentration time curve from time zero to the last measurable concentration time point; AUC_{0-∞} = Area under the plasma concentration time curve from time zero to infinity; C_{max} = maximum concentration observed after dosing.

[1037] Dose Pair-Wise Test Using Analysis of Variance
Table 11-38 presents pair-wise comparisons of single-dose PK parameters using ANOVA after dose normalizing. Results found that there were differences in the dose normalized values for all analytes for at least 1 of the 3 pair-wise comparisons except for free testosterone AUC measures for AUC₀₋₂₄, AUC₀₋₈, and AUC_{0-t}.

TABLE 11-38

Analysis of Variance for Some Pharmacokinetic Parameters (Single-Dose Population)						
Analyte	PK Parameter	Intra-subject Coefficient of Variation ^a	P-Value			
			600 µg vs. 1200 µg	600 µg vs. 1800 µg	1200 µg vs. 1800 µg	
Free Testosterone	AUC ₀₋₂₄	45.8505	0.2114	0.2182	0.9845	
	AUC ₀₋₈	67.4794	0.7110	0.5338	0.7996	
	AUC _{0-∞}	43.0039	0.0324	0.0197	0.8185	
	AUC _{0-t}	45.3344	0.2439	0.2477	0.9921	
	C_{max}	-223.3208	0.0034	0.0010	0.6182	
	λ_z	84.7568	0.0908	0.0406	0.6863	
	$t_{1/2}$	55.9189	0.0002	<0.001	0.3257	
Total Testosterone	AUC ₀₋₂₄	7.8951	<0.001	<0.001	0.0001	
	AUC ₀₋₈	8.2332	<0.001	<0.001	0.0001	
	AUC _{0-∞}	8.7190	<0.001	<0.001	0.0002	
	AUC _{0-t}	7.8792	<0.001	<0.001	0.0001	
	C_{max}	17.9163	<0.001	<0.001	0.0320	
	λ_z	85.1223	0.1153	0.0454	0.6333	
	$t_{1/2}$	55.6641	0.0002	<0.001	0.3940	
Dihydro-testosterone	AUC ₀₋₂₄	19.8167	<0.001	<0.001	0.1756	
	AUC ₀₋₈	23.5230	<0.001	<0.001	0.3291	
	AUC _{0-∞}	26.0151	0.0048	0.0004	0.3131	
	AUC _{0-t}	26.5016	0.0011	0.0001	0.3144	
	C_{max}	37.3379	0.0021	0.0009	0.6763	

TABLE 11-38-continued Analysis of Variance for Some Pharmacokinetic Parameters (Single-Dose Population)						
Analyte	PK Parameter	Intra-subject Coefficient of Variation ^a	P-Value			
			600 µg vs. 1200 µg	600 µg vs. 1800 µg	1200 µg vs. 1800 µg	
	λ_z	158.0250	0.1869	0.1523	0.9553	
	$t_{1/2}$	117.5657	0.2274	0.1041	0.7665	

AUC₀₋₈ = Area under the plasma concentration time curve from time zero to 8 hours after dosing;
AUC₀₋₂₄ = Area under the plasma concentration time curve from time zero to 24 hours after dosing;
AUC_{0-t} = Area under the plasma concentration time curve from time zero to the last measurable concentration time point;
AUC_{0-∞} = Area under the plasma concentration time curve from time zero to infinity;
 C_{max} = maximum concentration observed after dosing;
 λ_z = terminal elimination rate constant;
 $t_{1/2}$ = elimination half-life a Coefficient of Variation = 100 × ANOVA residual error (Root Mean Square Error)/PK parameter mean.
Pair-wise comparisons are from the ANOVA model with Dose as a class (categorical) variable and PK parameter estimate values as response.
Note:
AUC₀₋₈, AUC_{0-∞}, AUC_{0-t} and C_{max} parameter values are natural logarithmic transformed.

[1038] Paired t-Test Comparison of AUC₀₋₈ and AUC₀₋₂₄ from Single to Multiple Dose

[1039] There were 3 subjects who participated in both the single- and multi-dose periods at the 1200 µg dose. Uncorrected parameters for AUC₀₋₈ and AUC₀₋₂₄ were calculated from the free testosterone, total testosterone, and dihydrotestosterone analyte concentrations for comparison to uncorrected parameters from the multi-dose profile. Table 11-39 compares the AUC₀₋₈ and AUC₀₋₂₄ from these 3 subjects by using a paired t-test for free testosterone, total testosterone, and dihydrotestosterone. The results of the log transformed comparisons are provided.

TABLE 11-39

Paired t-Test Results for Pharmacokinetic Parameters AUC ₀₋₈ and AUC ₀₋₂₄ for Subjects Who Had 1200 µg TBS-2 in Period 1 and Period 2							
Analyte	Natural-logarithmic transformed PK Parameter		Mean of Difference	Standard Error of Difference	95% Confidence Limit for Mean Difference		P-Value
	Name	N			Lower Limit	Upper Limit	
Free Testosterone	AUC ₀₋₈	3	0.6041	0.4255	-1.2267	2.4349	0.2915
	AUC ₀₋₂₄	3	0.9312	0.3853	-0.7266	2.5890	0.1369
Total Testosterone	AUC ₀₋₈	3	0.5782	0.1780	-0.1876	1.3440	0.0831
	AUC ₀₋₂₄	3	0.9058	0.1683	0.1815	1.6301	0.0328
Dihydrotestosterone	AUC ₀₋₈	3	2.1025	0.2060	1.2160	2.9889	0.0095
	AUC ₀₋₂₄	3	2.1709	0.4044	0.4311	3.9108	0.0330

AUC₀₋₈ = Area under the plasma concentration time curve from time zero to 8 hours; AUC₀₋₂₄ = Area under the plasma concentration time curve from time zero to 24 hours; PK = pharmacokinetic.

[1040] The free testosterone comparisons of 95% CI included zero in the difference for both AUC measures so equivalence between the single- and multi-dose AUC measures could not be rejected. Total testosterone 95% CIs included zero for the AUC_{0-8} but not for the AUC_{0-24} so equivalence could not be rejected for AUC_{0-8} but was for AUC_{0-24} . In the analyte dihydrotestosterone, neither 95% CI included zero, so equivalence was rejected for both AUCs.

Statistical and Analytical Issues

[1041] K. Adjustments for Covariate

[1042] Not applicable.

[1043] L. Handling of Dropouts or Missing Data

To handle missing or partial AE and concomitant medication dates, the following rules were applied:

[1044] 1. For partial AE and concomitant medication start dates, if the year was unknown, then a missing value was assigned. If the month was unknown and the year matches the year of the first dose date, then the month and day of the first dose date was imputed; otherwise January was assigned. If the day was unknown and the month and year matched the month and year of the first dose date, then the day of the first dose date was imputed; otherwise "01" was assigned.

[1045] 2. For partial AE and concomitant medication end dates, if the year was unknown, then a missing value was assigned. If the month was unknown, then "December" was assigned. If the day was unknown, then the last day of the month was assigned.

[1046] After implementing the rules above, to determine whether AEs (or medications) with missing start or stop dates are pretreatment or on/after treatment, the following strategy was used:

[1047] 1. If the start date and stop date were both missing, then the most conservative approach was taken and the AE (or medication) was considered to be treatment-emergent (or concomitant).

[1048] 2. If the start date was missing but the stop date was not missing and was after the day of study dose administration, then the most conservative approach was taken and the AE (or medication) was considered to be treatment-emergent (or concomitant).

[1049] 3. If the start date was missing but the stop date was not missing and was on or before the day of study dose and after the date of signed informed consent, then the AE (or medication) was considered to be pretreatment (or prior).

[1050] 4. If the start date was not missing but the stop date was missing, then the most conservative approach was taken and medication was considered to be concomitant while the AE was defined by start date.

[1051] The missing severity of an AE was imputed to the greatest severity; the missing study drug causality to an AE was imputed to "related."

[1052] M. Interim Analysis and Data Monitoring

[1053] Interim analyses are discussed in the Interim Analysis section above.

[1054] N. Multi-Center Studies

[1055] Not applicable

[1056] O. Multiple Comparison/Multiplicity

[1057] A few pair-wise comparisons were performed, but no Type 1 error rate was adjusted since they were exploratory analyses.

[1058] P. Use of a "Pharmacokinetics Subset" of Subjects

[1059] All subjects were included in the PK analyses.

[1060] Q. Active-Control Studies Intended to Show Equivalence

[1061] Not applicable.

[1062] R. Examination of Subgroups

[1063] Not applicable.

Tabulation of Individual Response Data

[1064] Not applicable.

Drug Dose, Drug Concentration, and Relationship to Response

[1065] Not applicable.

Drug-Drug and Drug-Disease Interactions

[1066] Not applicable.

By-Subject Displays

[1067] Not applicable.

Pharmacokinetics Conclusions

[1068] 1. The single-dose baseline-corrected free and total testosterone concentrations have the clearest relationship of concentration to increasing testosterone dose. The dihydrotestosterone analyte concentrations provide a clear distinction between the 1800 μ g dose compared with the lower doses, which are not clearly differentiated from each other. A slight change is observed in the estradiol concentrations where it is the lowest dose of 600 μ g, which is clearly distinct from the two higher doses, which are not clearly differentiated from each other. The SHBG concentrations are not clearly distinguished between administered testosterone doses.

[1069] 2. The multi-dose concentrations were not baseline corrected but have similar graphical evaluations. The free and total testosterone concentrations have the clearest demonstration of the concentration increase due to dose. A more modest increase in dihydrotestosterone concentration is observed at the time of dose, but little increase in concentration of estradiol and SHBG is seen at the time of dose. When given BID at these dose, Testosterone accumulates 2-4 fold, but returned to baseline after 12-15 hours

[1070] 3. Pharmacokinetic parameters were listed individually and summarized for single-dose and multi-dose profiles. Formal statistical testing of dose proportionality was performed on the single-dose profiles.

[1071] 4. Testing of the single-dose profiles using linear regression across all 3 doses indicated that the free testosterone PK parameters AUC_{0-8} , AUC_{0-24} , and AUC_{0-t} were the only analyte parameters for which dose proportionality was not rejected.

[1072] 5. Pair-wise testing of doses from the single-dose profile using ANOVA agreed with the linear regression and also indicated that the free testosterone AUC_{0-8} , AUC_{0-24} , and AUC_{0-t} parameters were the only analyte parameters that indicated for which dose proportionality could not be rejected for at least one dose comparison.

[1073] 6. The 3 subjects who participated in both the single- and multi-dose profiles at the same 1200 μ g dose level were compared using a paired t-test for AUC_{0-8} and AUC_{0-24} . Equivalence between the uncorrected single- and multi-

dose profiles was rejected for both parameters for dihydrotestosterone, and for AUC₀₋₂₄ with total testosterone, but equivalence between the profiles was not rejected for either of the free testosterone parameters.

12. Safety Evaluation

[1074] A. Extent of Exposure

[1075] Study drug administration was performed at the clinical site under study personnel supervision

[1076] B. Adverse Events

Summary of Treatment Emergent Adverse Events

[1077] There were no deaths, SAEs, or AEs leading to discontinuation during the study. Most TEAEs were in the system organ classes of general disorders (nasal related events) and administration site conditions and respiratory, thoracic, and mediastinal disorders. Most TEAEs were mild in severity and were unlikely or not related to study medication.

Display of Treatment Emergent Adverse Events

[1078] The incidence of TEAEs is shown for Period 1 in Table 12-1 and Table 12-2.

TABLE 12-1

System Organ Class/ Preferred Term (MedDRA)	Cohort 1 (600 µg) (n = 8)	Cohort 2 (1200 µg) (n = 8)	Cohort 3 (1800 µg) (n = 8)	Total (N = 24)
Total Number of Subjects with at least 1 TEAE	4 (50.0%)	4 (50.0%)	3 (37.5%)	11 (45.8%)
Gastrointestinal disorders	1 (12.5%)	0	0	1 (4.2%)
Abdominal pain upper	1 (12.5%)	0	0	1 (4.2%)
General disorders and administration site conditions	3 (37.5%)	2 (25.0%)	2 (25.0%)	7 (29.2%)
Catheter site erythema	1 (12.5%)	1 (12.5%)	1 (12.5%)	3 (12.5%)
Catheter site hemorrhage	0	0	2 (25.0%)	2 (8.3%)
Catheter site inflammation	2 (25.0%)	0	0	2 (8.3%)
Catheter site pain	0	1 (12.5%)	0	1 (4.2%)
Infections and infestations	1 (12.5%)	0	0	1 (4.2%)
Vulvitis	1 (12.5%)	0	0	1 (4.2%)
Nervous system disorders	0	1 (12.5%)	1 (12.5%)	2 (8.3%)
Dizziness	0	1 (12.5%)	1 (12.5%)	2 (8.3%)
Headache	0	1 (12.5%)	0	1 (4.2%)
Respiratory, thoracic, and mediastinal disorders	1 (12.5%)	3 (37.5%)	1 (12.5%)	5 (20.8%)
Nasal congestion	0	2 (25.0%)	0	2 (8.3%)
Nasal mucosal disorder	0	1 (12.5%)	0	1 (4.2%)
Rhinalgia	0	0	1 (12.5%)	1 (4.2%)
Rhinorrhea	1 (12.5%)	0	0	1 (4.2%)

MedDRA = Medical Dictionary for Regulatory Activities;
TEAE = treatment-emergent adverse event

Note:

Subjects reporting more than 1 TEAE in each level (system organ class or preferred term) were only counted once.

TABLE 12-2

System Organ Class/ Preferred Term (MedDRA)	Total (N = 8)
Total Number of Subjects with at least 1 TEAE	4 (50.0%)
Gastrointestinal disorders	1 (12.5%)
Dyspepsia	1 (12.5%)
General disorders and administration site conditions	2 (25.0%)
Catheter site pain	1 (12.5%)
Catheter site phlebitis	1 (12.5%)
Nervous system disorders	1 (12.5%)
Headache	1 (12.5%)
Respiratory, thoracic, and mediastinal disorders	1 (12.5%)
Rhinalgia	1 (12.5%)

MedDRA = Medical Dictionary for Regulatory Activities;
TEAE = treatment-emergent adverse event

Note:

Subjects reporting more than 1 TEAE in each level (system organ class or preferred term) were only counted once.

Analysis of Adverse Events

[1079] Adverse Events in Period 1

[1080] Overall, 11 of 24 subjects (45.8%) experienced at least one TEAE in Period 1. Most subjects experienced TEAEs of general disorders and administration site conditions (experienced by 7 of 24 subjects [29.2%]) and respiratory, thoracic, and mediastinal disorders (experienced by 5 of 24 subjects [20.8%]). TEAEs experienced by more than 1 subject were catheter site erythema (experienced by 3 of 24 subjects [12.5%]) and catheter site hemorrhage, catheter site inflammation, dizziness, and nasal congestion (each experienced by 2 of 24 subjects [8.3%]). (Table 12-1 Table).

[1081] The majority of TEAEs in Period 1 were of mild severity. A total of 10 of 24 subjects (41.7%) experienced mild TEAEs. Only 1 of 24 subjects (4.2%) experienced moderate TEAEs. Subject 522-53 (Cohort 2) experienced headache and dizziness of moderate severity on Day 1. Both TEAEs resolved without treatment the next day and were considered as unlikely to be related to the study drug.

[1082] Overall, 4 of 24 subjects (16.7%) experienced TEAEs that were considered possibly related to the study medication. A total of 3 of 24 subjects (12.5%) had TEAEs that were considered to be unlikely related to the study medication and 4 of 24 subjects (16.7%) had TEAEs that were considered not related to the study medication.

[1083] Adverse Events in Period 2

[1084] Overall, 4 of 8 subjects (50.0%) experienced at least 1 TEAE in Period 2. General disorders and administration site conditions occurred in 2 of 8 subjects (25.0%). The TEAEs that were experienced by 1 of 8 subjects (12.5%) each were catheter site pain, catheter site phlebitis, dyspepsia, headache, and rhinalgia. (Table 12-2).

[1085] The majority of TEAEs in Period 2 were of mild severity. Overall, 3 of 8 subjects (37.5%) experienced mild TEAEs. Only 1 of 8 subjects (12.5%) experienced a moderate TEAE. Subject 522-29 experienced an increase in headache intensity of moderate severity on Day 2. The TEAE resolved without treatment 2 days later and was considered as possibly related to the study drug.

[1086] Overall, 2 of 8 subjects (25.0%) experienced TEAEs that were considered possibly related to the study medication

and 2 of 8 subjects (25.0%) experienced TEAEs that were considered not related to the study medication.

[1087] Adverse Reactions in Period 1 and Period 2

[1088] Adverse reactions (defined as TEAEs considered possibly, probably, or definitely related to study medication) in Period 1 and Period 2 are shown in Table 12-3.

[1089] A total of 6 subjects experienced adverse reactions during the study: 4 of 24 subjects (16.7%) in Period 1 and 2 of 8 subjects (25.0%) in Period 2. All adverse reactions were considered possibly related to study medication. Subject 522-29 (Period 2) had 2 adverse reactions of headache that were each recorded as a TEAE: a mild headache that increased in severity to moderate.

TABLE 12-3

Subjects with Adverse Reactions (Single- and Multi-Dose Populations)				
Subject	Preferred Term (MedDRA)	Onset ^a	Severity ^b	Outcome
Period 1, 600 µg				
522-37	Rhinorrhea	Day 2	Mild	Resolved
Period 1, 1200 µg				
522-35	Nasal congestion	Day 1	Mild	Resolved
522-51	Nasal congestion	Day 1	Mild	Resolved
522-53	Nasal mucosal disorder	Day 3	Mild	Ongoing
Period 21200 µg				
522-29	Headache	Day 2	Mild	Resolved
	Headache (increase in intensity)	Day 2	Moderate	Resolved
522-24	Rhinalgia	Day 2	Mild	Resolved

MedDRA = Medical Dictionary for Regulatory Activities;

TEAE = treatment-emergent adverse event

^aThe onset was calculated, respectively, as [Onset Date – Treatment Date of Period 1 + 1], if the onset was during Period 1, or [Onset Date – Treatment Date of Period 2 + 1], if the onset was during Period 2.

^bSeverity was rated by the Principal Investigator as mild, moderate, or severe.

Listing of Adverse Events by Subject

[1090] Adverse events are listed by subject in Appendix 16.2, Listing 16.2.7.1. Adverse reactions (defined as TEAEs considered possibly, probably, or definitely related to study medication) in Period 1 and Period 2 are listed by subject in Appendix 16.2, Listing 16.2.7.4.

[1091] C. Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

[1092] There were no deaths, SAEs, or AEs leading to discontinuation during the study.

[1093] D. Clinical Laboratory Evaluations

Valuation of Each Laboratory Parameter

[1094] Laboratory Values Over Time

[1095] Changes over time in laboratory values were not calculated.

[1096] Individual Subject Changes

[1097] Subjects with abnormal laboratory values that were not present at screening are shown in Table 12-4 (hematology), Table 12-5 (chemistry), and Table 12-26 (urinalysis).

TABLE 12-24

Subjects with New Abnormal Hematology Laboratory Evaluation Results Post Dose (Single- and Multi-Dose Populations)					
Subject	Study Period	Study Day	Test (Normal Range)	Result	Low/High
Cohort 1, 600 µg					
522-15	1	4	Hematocrit (32.5%-46.9%)	32.0%	Low
	1	4	Red Blood Cell Count (3.70-5.46 mil/mm ³)	3.55 mil/mm ³	Low
522-40	1	4	Lymphocytes (15.0%-50.0%)	50.6%	High
522-49	1	4	Absolute Basophil Count (0-125 cells/mm ³)	140 cells/mm ³	High
Cohort 2, 1200 µg					
522-28	2	5	Absolute Basophil Count (0-125 cells/mm ³)	160 cells/mm ³	High
	2	5	Basophil Percentage (0.0-2.0%)	2.6%	High
522-38	2	5	Absolute Basophil Count (0-125 cells/mm ³)	160 cells/mm ³	High
Cohort 3, 1800 µg					
522-24	1	4	Eosinophils Percentage (0.0%-6.0%)	7.0%	High

TABLE 12-25

Subjects with New Abnormal Chemistry Laboratory Evaluation Results Post Dose (Single- and Multi-Dose Populations)					
Subject	Study Period	Study Day	Test (Normal Range)	Result	Low/High
Cohort 1, 600 µg					
522-03	1	4	Potassium (3.2-5.2 mmol/L)	5.4 mmol/L	High
522-29	1	4	Prolactin (1.9-25.0 ng/mL)	27.0 ng/mL	High
522-40	1	4	TSH (0.40-4.00 uIU/mL)	4.34 uIU/mL	High
	1	4	Total T3 (84-172 ng/dL)	83 ng/dL	Low
522-43	1	4	Prolactin (1.9-25.0 ng/mL)	36.8 ng/mL	High
Cohort 2, 1200 µg					
522-09	1	4	Prolactin (1.9-25.0 ng/mL)	31.7 ng/mL	High
522-10	1	4	Total T3 (84-172 ng/dL)	72 ng/dL	Low
522-28	1	4	Total T3 (84-172 ng/dL)	72 ng/dL	Low
	2	5	Prolactin (1.9-25.0 ng/mL)	37.1 ng/mL	High
	2	5	Free T4 (0.89-1.76 ng/dL)	0.83 ng/dL	Low
	2	5	Total T3 (84-172 ng/dL)	78 ng/dL	Low

TABLE 12-25-continued

Subjects with New Abnormal Chemistry Laboratory Evaluation Results Post Dose (Single- and Multi-Dose Populations)					
Subject	Study Period	Study Day	Test (Normal Range)	Result	Low/High
522-34	1	4	Prolactin (1.9-25.0 ng/mL)	33.2 ng/mL	High
522-38	1	4	CK (33-211 U/L)	32 U/L	Low
	2	5	Free T4 (0.89-1.76 ng/dL)	0.76 ng/dL	Low
522-51	1	4	Fasting Glucose (70-99 mg/dL)	101 mg/dL	High
522-53	1	4	Prolactin (1.9-25.0 ng/mL)	56.3 ng/mL	High
Cohort 3, 1800 µg					
522-01	1	4	CK (33-211 U/L)	26 U/L	Low
	1	4	Free T4 (0.89-1.76 ng/dL)	0.82 ng/dL	Low
	2	5	CK (33-211 U/L)	28 U/L	Low
	2	5	Total T3 (84-172 ng/dL)	77 ng/dL	Low
522-17	1	4	Prolactin (1.9-25.0 ng/mL)	30.4 ng/mL	High
	2	5	Prolactin (1.9-25.0 ng/mL)	40.5 ng/mL	High
522-18	1	4	Prolactin (1.9-25.0 ng/mL)	25.8 ng/mL	High
	1	4	Total T3 (84-172 ng/dL)	73 ng/dL	Low
522-42	1	4	Prolactin (1.9-25.0 ng/mL)	31.1 ng/mL	High
522-44	1	4	Prolactin (1.9-25.0 ng/mL)	29.9 ng/mL	High
	1	4	TSH (0.40-4.00 uIU/mL)	6.02 uIU/mL	High
522-46	1	4	Prolactin (1.9-25.0 ng/mL)	45.0 ng/mL	High
	1	4	Total T3 (84-172 ng/dL)	66 ng/dL	Low
522-54	1	4	Prolactin (1.9-25.0 ng/mL)	57.2 ng/mL	High
	1	4	Free T4 (0.89-1.76 ng/dL)	0.81 ng/dL	Low

CK = creatine kinase;

TSH = thyroid-stimulating hormone

TABLE 12-26

Subjects with New Abnormal Urinalysis Laboratory Evaluation Results Post Dose (Single- and Multi-Dose Populations)					
Subject	Study Period	Study Day	Test (Normal Range)	Result	Low/High
Cohort 1, 600 µg					
522-15	1	4	Blood (Negative)	1+	High
522-29	1	4	Blood (Negative)	Trace	High
	2	5	Blood (Negative)	Trace	High
522-52	1	4	Blood (Negative)	Trace	High
Cohort 2, 1200 µg					
522-28	1	4	Blood (Negative)	3+	High
522-35	2	5	Protein (Negative or Trace)	1+	High
522-38	2	5	Blood (Negative)	Trace	High
522-51	1	4	Blood (Negative)	1+	High
	1	4	Leukocyte Esterase (Negative)	3+	High
Cohort 3, 1800 µg					
522-17	1	4	Blood (Negative)	1+	High
	2	5	Blood (Negative)	Trace	High
522-18	1	4	Blood (Negative)	Trace	High
522-24	2	5	Blood (Negative)	1+	High

[1098] Individual Clinically Significant Abnormalities**[1099]** No abnormal clinical laboratory results were recorded as TEAEs.**[1100]** E. Vital Signs, Physical Examinations, and Other Observations Related to Safety**Vital Signs****[1101]** No abnormal vital signs were reported by the PI or were recorded as a TEAE.**Physical Examination****[1102]** Three subjects had abnormal physical examination results related to the PK blood sample venipuncture site. None of these results were considered clinically significant.**[1103]** Subject 522-35 (1200 µg)—Period 2, Day 5: Slight phlebitis to left antecubital site**[1104]** Subject 522-38 (1200 µg)—Period 2, Day 5: Tenderness/soreness on left antecubital area**[1105]** Subject 522-51 (1200 µg)—Period 1, Day 4: Slight tenderness left AC IV site**[1106]** Except for those related to HEENT or the PK blood sample venipuncture site, no other abnormal physical examination results were reported.**Nasal Endoscopic Examination****[1107]** No ENT nasal endoscopic examination findings were reported at Screening (Appendix 16.2, Listing 16.2.4.3. 2).**[1108]** Two subjects had ENT examination findings on Day 4 in Period 1 that were interpreted as clinically significant by the PI: Subject 522-37 (600 µg) had slight rhinorrhea and Subject 522-53 (1200 µg) had mild erythema to the left nostril mucosa.**[1109]** All abnormal findings in basic ENT examinations are presented in Table 12-27.

TABLE 11-27

Subjects with Abnormal Basic Ear, Nose, and Throat Examination Results (Single- and Multi-Dose Populations)				
Subject	Study Period	Study Day	Test Comments	Investigator Interpretation
Cohort 1, 600 µg				
522-03	2	1	Slight erythema	Abnormal NCS
522-37	1	1	Slight edema of turbinates	Abnormal NCS
	1	4	Slight rhinorrhea	Abnormal CS ^a
522-43	1	1	Slight erythema of turbinates. Nasal passages are patent.	Abnormal NCS

TABLE 11-27-continued

Subjects with Abnormal Basic Ear, Nose, and Throat Examination Results (Single- and Multi-Dose Populations)				
Subject	Study Period	Study Day	Test Comments	Investigator Interpretation
	1	4	Minimal less erythema than at check in	Abnormal NCS
522-52	1	1	Slight erythema Cohort 2, 1200 µg	Abnormal NCS
522-35	1	1	Very slight edema left turbinates	Abnormal NCS
522-38	2	1	Slight erythema, probably temperature related	Abnormal NCS
522-51	1	1	Mild erythema to mucosa	Abnormal NCS
	1	4	Very minimal erythema, no edema	Abnormal NCS
522-53	1	4	Erythema to left nostril slightly more than right, mucosa of left slightly erythematous relative to right, noticeable. Cohort 3, 1800 µg	Abnormal CS ^b
522-17	1	4	Appears to be slight edema of right mucosa and turbinates relative to left. The nostril is patent so likely not clinically significant.	Abnormal NCS
	2	5	Appears to be slight edema of right mucosa and turbinates relative to left. The nostril is patent so likely not clinically significant.	Abnormal NCS
522-24	1	1	Slight edema turbinates on right but minimal	Abnormal NCS

CS = clinically significant;

ENT = ears, nose, and throat;

NCS = not clinically significant;

TEAE = treatment emergent adverse event

^aA TEAE of rhinorrhea (mild, possibly related to study medication) was reported on Day 2 and resolved on Day 5.^bA TEAE of nasal mucosal disorder (mild, possibly related to study medication) was reported on Day 3 and was ongoing at study completion.**[1110] F. Safety Conclusions**

[1111] There were no deaths, SAEs, or AEs leading to discontinuation during the study. Most TEAEs were in the system organ classes of general disorders and administration site conditions and respiratory, thoracic, and mediastinal disorders (nasal related events). Most TEAEs were mild in severity and were unlikely or not related to study medication.

[1112] In Period 1, a total of 11 of 24 subjects (45.8%) experienced at least 1 TEAE. Those experienced by more than 1 subject were catheter site erythema (experienced by 3 of 24 subjects [12.5%]) and catheter site hemorrhage, catheter site inflammation, dizziness, and nasal congestion (each experienced by 2 of 24 subjects [8.3%]).

[1113] The majority of TEAEs in Period 1 were of mild severity. A total of 10 of 24 subjects (41.7%) experienced mild TEAEs. Only 1 of 24 subjects (4.2%) experienced moderate TEAEs. Subject 522-53 (1200 µg) experienced headache and dizziness of moderate severity on Day 1. Both TEAEs resolved without treatment the next day and were considered as unlikely to be related to the study drug.

[1114] In Period 2, a total of 4 of 8 subjects (50.0%) experienced at least 1 TEAE. General disorders and administration site conditions occurred in 2 of 8 subjects (25.0%). The TEAEs that were experienced by 1 of 8 subjects (12.5%) each were catheter site pain, catheter site phlebitis, dyspepsia, headache, and rhinalgia.

[1115] The majority of TEAEs in Period 2 were of mild severity. Overall, 3 of 8 subjects (37.5%) experienced mild TEAEs. Only 1 of 8 subjects (12.5%) experienced a moderate TEAE. Subject 522-29 experienced an increase in headache intensity of moderate severity on Day 2. The TEAE resolved without treatment 2 days later and was considered as possibly related to the study drug.

[1116] A total of 6 subjects experienced adverse reactions (defined as TEAEs considered possibly, probably, or definitely related to study medication) during the study; 4 subjects in Period 1 and 2 subjects in Period 2. All adverse reactions were considered possibly related to study medication. Subject 522-29 (Period 2, 600 µg) had 2 adverse reactions of headache that were each recorded as a TEAE: a mild headache that increased in severity to moderate.

[1117] A total of 6 subjects experienced adverse reactions during the study: 4 of 24 subjects (16.7%) in Period 1 and 2 of 8 subjects (25.0%) in Period 2. All adverse reactions were considered possibly related to study medication. Subject 522-29 (Period 2) had 2 adverse reactions of headache that were each recorded as a TEAE: a mild headache that increased in severity to moderate.

[1118] There were no TEAEs associated with clinical laboratory tests or vital signs. Two subjects had ENT examination findings that were interpreted as clinically significant by the PI on Day 4 in Period 1: Subject 522-37 (600 µg) had slight rhinorrhea and Subject 522-53 (1200 µg) had mild erythema to the left nostril mucosa.

13. CMC Section

[1119] Testosterone batch, 80402960, used in the clinical batches was fully tested by the applicant in accordance with USP specifications.

[1120] The drug product, TBS-2 is a viscous bioadhesive oil-based formulation containing solubilized testosterone intended for intranasal application for the treatment of anorgasmia in women.

[1121] The drug product is formulated with the following compendial inactive ingredients: castor oil, oleoyl polyoxyglycerides, and colloidal silicon dioxide. TBS-2 is supplied in a white, non-aerosol, multi-dose, metered pump container. The containers are supplied separately as barrels (Albion 15 ml) and pumps (VP39/140H) with cap attached. The container closure is manufactured using materials acceptable for pharmaceutical use. See FIG. 39.

[1122] The container closure relies on atmospheric pressure and device design to deliver the required dose. When the actuator of the VP39/140 pump is pressed, a valve is opened in the pump mechanism. This allows atmospheric pressure to act on the piston, through the base of the gel-containing Albion 15 ml barrel, forcing it upwards. Consequently, the gel is forced upwards, through the tip of the device. The accurate and concise quantity of gel delivered is a function of the design of VP39/140 pump. When the actuation is stopped, a spring in the pump mechanism closes the valve, stops the effect of atmospheric pressure on the piston and returns the actuator to its starting position.

[1123] The patient is instructed to place his finger on the pump of the actuator and to advance the tip of the actuator until the finger on the pump reaches the base of the nose. The opening on the tip of the actuator must face the nasal mucosa. The patient depresses the pump and the gel is expelled onto the nasal mucosa. Each dose consists of two actuations, one actuation per nostril.

[1124] The compositions of the three different concentrations of the drug product to be used in this clinical trial are provided in Tables 3.2.P.1-1-3.

TABLE 3.2.P.1-1

Components, Quality, Quality Standards and Function - 0.24% TBS-2 Gel					
Component	Amount (% w/w)	Amount per multiple dose dispenser (g)	Amount delivered per actuation (mg)	Function	Quality Standard
Testosterone	0.24	0.024	0.30	Active Ingredient	USP
Castor oil	91.76	9.176	114.70	Solvent	USP
Oleoyl polyoxyglycerides	4.00	0.400	5.00	Wetting agent (hydrophilic oil)	Ph. Eur./NF
Colloidal silicon dioxide	4.00	0.400	5.00	Viscosity increasing agent	NF
Total	100.00	10.0	125.00		

TABLE 3.2.P.1-2

Components, Quality, Quality Standards and Function - 0.48% TBS-2 Gel					
Component	Amount (% w/w)	Amount per multiple dose dispenser (g)	Amount delivered per actuation	Function	Quality Standard
Testosterone	0.48	0.048	0.60	Active Ingredient	USP
Castor oil	91.52	9.152	114.40	Solvent	USP
Oleoyl polyoxyglycerides	4.00	0.400	5.00	Wetting agent (hydrophilic oil)	Ph. Eur./NF
Colloidal silicon dioxide	4.00	0.400	5.00	Viscosity increasing agent	NF
Total	100.00	10.0 g	125.00		

TABLE 3.2.P.1-3

Components, Quality, Quality Standards and Function - 0.72% TBS-2Gel					
Component	Amount (% w/w)	Amount per multiple dose dispenser (g)	Amount delivered per actuation (mg)	Function	Quality Standard
Testosterone	0.72	0.072	0.90	Active Ingredient	USP
Castor oil	91.28	9.128	114.10	Solvent	USP
Oleoyl polyoxyglycerides	4.00	0.400	5.00	Wetting agent (hydrophilic oil)	Ph. Eur./NF
Colloidal silicon dioxide	4.00	0.400	5.00	Viscosity increasing agent	NF
Total	100.00	10.0	125.00		

[1125] A study is completed in accordance with USP<51> Antimicrobial Effectiveness Testing to establish that microbial growth is absent in the container closure system after filling and during use. This study is performed with a 4.5% testosterone gel filled in the multiple dose dispenser.

[1126] Sixteen (16) multiple dose dispensers are tested in this study. Each multiple dose dispensers is inoculated with 0.05 mL of one of the standardized microbial suspensions (*Candida albicans*, *Aspergillus brasiliensis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) through the tip of the piston. These microorganisms are chosen as they are the microorganisms recommended in USP <51> Antimicrobial Effectiveness Testing for nasal products. The theoretical concentration of the organisms used for the inoculation is between 100,000 and 1,000,000 microorganisms per mL. For the following 21 days, two actuations are performed on each multiple dose dispenser daily to reflect real use (2 actuations is equivalent to 1 dose).

[1127] The inoculated dispensers are incubated upright at 20-25° C. Each of the samples is examined at 1, 7, 14 and 21 days, subsequent to inoculation. The study is conducted over twenty-one (21) days. The Day 1 timepoint is chosen to assess the worst case scenario. Testing of the samples does occur following the daily actuations. For all the timepoints, the gel remaining in the barrel is tested. The piston is removed and the remaining gel in the barrel was subjected to further testing to determine the number of test organisms present, using 10 grams of the sample. The sample is examined at these intervals for any observed changes in appearance (i.e. colour, viscosity). Each dispenser is discarded after sampling. The results are presented in Table 3.2.P.2.5-1.

TABLE 3.2.P.2.5-1

Number of Microorganisms Surviving During Testing Period					
Organism	Initial	Number of Microorganisms surviving during testing period			
	Count	Day 1	Day 7	Day 14	Day 21
	Microorganisms per gram				
<i>S. aureus</i>	535,000	Less than 10	Less than 10	Less than 10	Less than 10
	Log value 5.7	(ND)	(ND)	(ND)	(ND)
<i>P. aeruginosa</i>	405,000	Less than 10	Less than 10	Less than 10	Less than 10
	Log value 5.6	(ND)	(ND)	(ND)	(ND)
<i>C. albicans</i>	980,000	Less than 10	Less than 10	Less than 10	Less than 10
	Log value 6.0	(ND)	(ND)	(ND)	(ND)
<i>A. brasiliensis</i>	675,000	Less than 10	Less than 10	Less than 10	Less than 10
	Log value 5.8	(ND)	(ND)	(ND)	(ND)
		Log value <1.0	Log value <1.0	Log value <1.0	Log value <1.0

ND—none detected; No changes were observed in the appearance of the product at each timepoint during the testing interval

count at 14 days and no increase from the 14 days count at 21 days. The yeast *C. Albicans* and *A. Brasiliensis* showed no increase from the initial calculated count at 14 and 21 days. Testosterone nasal gel packaged in a multiple dose dispenser meets the criteria for the Antimicrobial Effectiveness Test.

[1129] Three different concentrations of TBS-2 clinical material, 0.24%, 0.48% and 0.72%, will be manufactured for the proposed clinical trial. The batch formulae for these batches are presented in Table 3.2.P.3.2-1.

TABLE 3.2.P.3.2-1

Batch Formulae for 0.24%, 0.48% and 0.72% TBS-2 at the 100 kg Batch Size			
Components	Quantity per Batch (kg)		
	0.24% TBS-2	0.48% TBS-2	0.72% TBS-2
Testosterone, USP	0.24	0.48	0.72
Castor oil, USP	91.76	91.52	91.28
Oleoyle polyoxyglycerides, Ph. Eur./NF	4.00	4.00	4.00
Colloidal silicon dioxide, NF	4.00	4.00	4.00
Total	100.00	100.00	100.00

The TBS-2 bulk gel is tested to the following specifications for batch release.

TABLE 3.2.P.2.5-2

Criteria for Tested Microorganisms (Category 2)	
Criteria for Tested Microorganisms (Category 2)	
Bacteria	Not less than 2.0 log reduction from the initial calculated count at 14 days, and no increase from the 14 day count at 28 days.
Yeast and Molds	No increase from the initial calculated count at 14 and 28 days

TABLE 3.2.P.5.1-1

Specification for TBS-2 Bulk Gel		
Test Parameter	Method/Reference	Acceptance Criteria
Appearance	STM.GEN.001	Slightly yellow gel
Identification A	STM.TBS2.001	Retention time of the peak in the sample corresponds to that of the Testosterone standard.
Identification B	STM.TBS2.001	UV spectrum of the sample corresponds to that of the Testosterone standard

[1128] In summary, the bacteria *S. Aureus* and *P. Aeruginosa* showed a reduction of not less than 2.0 from the initial

TABLE 3.2.P.5.1-1-continued

Specification for TBS-2 Bulk Gel			
Test Parameter	Method/ Reference	Acceptance Criteria	
Appearance	STM.GEN.001	Slightly yellow gel	
Assay	STM.TBS2.001	95.0-105.0%	
Related Compounds	STM.TBS2.002	TBS1 RC4	δ 0.2%
		TBS1 RC5	δ 0.5%
		Single unknown impurity	δ 0.2%
Viscosity	USP<911>	Total impurities	δ 1.0%
Water	USP<921>	Report results	
Residual Solvents	Method I USP<467>	The product complies with USP<467> requirements	

TBS1 RC4 - 17 β -hydroxyandrost-4,6-dien-3-one (Delta-6-testosterone);
 EP Impurity I TBS1 RC5 - 17 β -hydroxyandrost-4-en-3-one (Epitestosterone);
 EP Impurity C

[1130] The TBS-2 gel packaged in the multiple dose dispenser is tested to the following specifications for batch release.

TABLE 3.2.P.5.1-2

Specification for TBS-2 Gel Packaged in Multiple Dose Dispenser			
Test Parameter	Method/ Reference	Acceptance Criteria	
Appearance	STM.GEN.001	White barrel and cap, filled with slightly yellow gel	
Identification A	STM.TBS2.001	Retention time and spectrum corresponds to standard	
Identification B	STM.TBS2.001	UV spectrum matches reference spectrum	
Delivered Dose Uniformity	STM.TBS2.003	Meets USP <601> Mean (beginning doses & end doses) Min: Max: % RSD:	
Related Compounds	STM.TBS2.002	TBS1 RC4	δ 0.2%
		TBS1 RC5	δ 0.5%
		Single unknown impurity	δ 0.2%
Residual Solvents	USP<467>	Total impurities	δ 1.0%
Microbial	USP<61>& <62>	Complies with USP<467>	
Limits		Total aerobic microbial count	<10 ² cfu/g
		Total combined yeasts/ mould count	<10 cfu/g
		<i>P. aeruginosa</i>	0/g

TBS1 RC4 - 17 β -hydroxyandrost-4,6-dien-3-one (Delta-6-testosterone);
 EP Impurity I TBS1 RC5 - 17 β -hydroxyandrost-4-en-3-one (Epitestosterone);
 EP Impurity C

[1131] Delivered Dose Uniformity as per USP has been added as a release test to verify the performance of the dispenser. In addition, the analytical procedure to test for related compounds has been modified. The viscosity and water content of the gel are tested upon release for information purposes only. These analytical procedures are described in this section.

[1132] Ten individual barrels are taken and tested for Delivered Dose Uniformity according to the method summarized in Table 3.2.P.5.2.1.1-1. Each dispenser is primed by actuating the pump 10 times. The drug content of the first dose, i.e. actuations #11 and #12 and the final dose (based on label

claim of 10 doses, i.e. 20 actuations), i.e. actuation #29 and #30 are tested. The mean of the 10 doses (20 actuations) are reported.

TABLE 3.2.P.5.2.1.1-1

Summary of Chromatographic Conditions for Delivered Dose Uniformity	
Mobile Phase	Mobile Phase: Methanol:Water 60:40
Column	Type: Thermo ODS Hypersil Dimensions: 100 cm x 4.6 mm Particle Size: 5 μ m
Flow Rate	1.0 mL/min
Column Temp.	40° C.
Detector	245 nm
Injection Volume	5 μ L
Run Time	9 minutes
Retention Time (RT)	Testosterone Typical RT 5.0 min
Standard and Sample Concentrations	For Delivered Dose: 600 μ g/dose: Standard contains 15 μ g/mL of testosterone Sample contains of 15 μ g/mL of testosterone; 1200 μ g/dose & 1800 μ g/dose: Standard contains 30 μ g/mL of testosterone Sample contains of 30 μ g/mL of testosterone;

[1133] The related compounds TBS1 RC4 (Impurity I/ Δ -6-testosterone) and TBS1 RC5 (Impurity C/epitestosterone) in the finished product are analysed by HPLC, as well as the unknown impurities according to the method summarized in Table 3.2.P.5.2.1.2-1.

TABLE 3.2.P.5.2.1.2-1

Summary of Chromatographic Conditions for Related Compounds Method			
Mobile Phase	Mobile Phase A: Water, 100% Mobile Phase B: Acetonitrile:Methanol 60:40 Filter and de-aerate each mobile phase		
	Gradient Program		
	Time	Mobile Phase A	Mobile Phase B
	0	70	30
	5.0	70	30
	15.0	20	80
	20.0	0	100
	25.0	0	100
	30.0	70	30
Column	Type: Hypersil BDS C18 Dimensions: 250 cm × 4.0 mm Particle Size: 5 µm		
Flow Rate	1.0 mL/min		
Column Temp.	40° C.		
Detector	240 nm & 290 nm		
Injection Volume	20 µL		
Run Time	30 minutes		
Retention Time (RT)	Related Compound	Typical RT	
	Impurity I	14.0 min	
	Testosterone	14.6 min	
	Impurity C	16.0 min	
Standard and Sample Concentrations	Standard contains 0.75 µg/mL of testosterone and 2 µg/mL of Impurity I Sample contains of 48 µg/mL of testosterone		

[1134] The measurement of the viscosity of TBS-2 is performed using a rotational viscosimeter. The results are reported for information purposes.

[1135] The content of water in the gel is determined using a direct titration measurement as per USP Method 1a. The results of the water content are reported for information purposes.

[1136] Assay and Delivered Dose Uniformity as per USP has been added as a release test to verify the performance of the dispenser. In addition, the analytical procedure to test for related compounds has been modified. The validation of these analytical procedures are described in this section.

[1137] The Assay and Delivered Dose method has been validated according to the parameters listed in Table 3.2.P.5.3.1-1.

TABLE 3.2.P.5.3.1-1

Assay and Delivered Dose Validation				
Test	Acceptance Criteria	Results		
Linearity	$r^2 \geq 0.998$	$r^2 = 0.999$		
Accuracy	Mean Recovery	0.24% and 0.48% Gel		
	98-102%	Mean = 100.6% (for all levels		
	Individual 98-102%	50%, 100% and 150%) % RD = 0.6% 0.72% Gel		
System Precision	RSD $\leq 2.0\%$	Mean = 100.0% (for all levels		
		50%, 100% and 150%) % RD = 0.2%		
		At 15 µg/mL RSD 0.2% At 30 µg/mL RSD 0.4%		
Method Precision	Repeatability and Intermediate Precision Assay for 6 samples RSD $\leq 2.0\%$ Absolute Difference between Chemist 1 and Chemist 2 is NMT 2%	0.24% TBS-2		
		Chemist 1	Chemist 2	
		Mean	98.2%	98.2%
Method Precision	Repeatability and Intermediate Precision Assay for 6 samples RSD $\leq 2.0\%$ Absolute Difference between Chemist 1 and Chemist 2 is NMT 2%	RSD	0.1%	0.3%
		Absolute Difference	0.0	
		Absolute Difference		

TABLE 3.2.P.5.3.1-1-continued

Assay and Delivered Dose Validation				
Test	Acceptance Criteria	Results		
		0.48% TBS-2		
		Chemist 1	Chemist 2	
		Mean	96.6%	97.0%
		RSD	0.1%	0.2%
		Absolute Difference	0.4	
		0.72% TBS-2		
		Chemist 1	Chemist 2	
		Mean	99.0%	98.3%
		RSD	0.2%	0.1%
		Absolute Difference	0.7	
Stability of	Standard Solution for 0.24% and 0.48%	5 Days		
	Standard Solution for 0.72%	5 Days		
	Sample Solution for 0.24% and 0.48%	5 Days		
	Sample Solution for 0.72%	5 Days		
Selectivity	No interference of excipient mixture with testosterone peak	Complies		

[1138] Related Compounds/Degradation Products by HPLC. The method has been validated to the performance characteristics presented in Table 3.2.P.5.3.2-1 and 3.2.P.5.3.2-2.

TABLE 3.2.P.5.3.2-1

Analytical Validation for the Determination of Related Compounds by HPLC				
Test	Acceptance Criteria	Results		
		TBS1 RC4 (Limit: 0.2%)	Active (for unknown) (Limit: 0.2%)	
Linearity		Number of concentrations	7	7
		Range (expressed as % and conc µg/mL)	0.045 µg/mL to 2.252 µg/mL	0.045 µg/mL to 2.324 µg/mL
		Slope	62.402	103.3735
	$r^2 \geq 0.99$	Y-intercept	0.1604	0.1766
		Correlation coefficient (r ²)	1.00	1.00
LOQ	% RSD $\leq 10.0\%$ of six injections at the LOQ concentration	Concentration	0.1%	0.1%
		% RSD	1.4%	2.1%
System Precision		Conc. (s) (expressed as % "A")	100% (Standard)	100% (Standard)
		Number of replicates	6	6
		Result average %	80.12765	46.88562
Recovery At LOQ	Mean recovery is between 80 to 120%	RSD	0.2	0.2
		Number of replicates	3	3
		% Recovery at LOQ of specified limit	103.8	N/A
		% RSD at LOQ	2.5	

TABLE 3.2.P.5.3.2-1-continued

Analytical Validation for the Determination of Related Compounds by HPLC				
Test	Acceptance Criteria	Results		
			TBS1 RC4 (Limit: 0.2%)	Active (for unknown) (Limit: 0.2%)
Recovery	Mean	Number of replicates	3	3
At 100%	recovery is between 80 to 120%	% Recovery at 100% of specified limit	101.2	N/A
		% RSD	1.4	
Recovery	Mean	Number of replicates	3	3
At 150%	recovery is between 80 to 120%	% Recovery at 150% of specified limit	99.3	N/A
		% RSD	1.3	
Method	% RSD of	TBS1 RC4	TBS1 RC5	
Precision	mean result	Chemist 1	Chemist 1	
	values of	Mean = 0.20%	Mean = 0.24%	
	known related	% RSD = 1.3	% RSD = 1.4	
	compound	Chemist 2	Chemist 2	
	obtained from	Mean = 0.19%	Mean = 0.24%	
	six samples:	% RSD = 1.6	% RSD = 1.3	
	≤10%			

TABLE 3.2.P.5.3.2-2

Analytical Validation for the Determination of Related Compounds by HPLC		
Test	Acceptance Criteria	Results
Stability of Standard Solution	The recovery of the standard solution is 80 to 120%	Standard solution is stable for 40 hours at room temperature
Stability of Sample Solution	Sample solution: The recovery of the testosterone and TBS 1 RC4 solution is 80 to 120% No significant change in known, unknown and total % of RCs over the specified length of time determined	Sample solution is stable for 1 Day at room temperature
Selectivity	The analytes of interest are free of interference.	Conforms

[1139] It can be concluded that the analytical method meets the acceptance criteria described in the validation plan.

[1140] For the Phase I trial, three bulk gel batches of TBS-2 have been manufactured and are summarized in Table 3.2.P.5.4-1. Batch analysis data from the bulk gel batches IMP1

1005, IMP1 1006 and IMP1 1007 are presented in Tables 3.2.P.5.4-2 and batch analysis data from finished drug product batches IMP1 1008, IMP1 1009 and IMP1 1010 are presented in Table 3.2.P.5.4-3.

TABLE 3.2.P.5.4-1

Description of TBS-2 Batches			
Concentration	0.24%	0.48%	0.72%
Bulk Batch No.	IMP11005	IMP11006	IMP11007
Finished Product	IMP11008	IMP11009	IMP11010
Batch No.			
Batch No.	80402960	80402960	80402960
Testosterone			
Equipment	100 kg	100 kg	100 kg
Batch Size			
Filling Quantity per	10 g	10 g	10 g
Multiple Dose			
Dispenser1			

* Net fill weight 10.g ± 1.0 g

TABLE 3.2.P.5.4-2

Batch Analysis - TBS-2 Bulk Gel, Batches IMP1 1005, IMP1 1006 and IMP1 1007				
Test Parameter	Acceptance Criteria	IMP1 1005	IMP1 1006	IMP1 1007
Appearance	Slightly yellow gel	Complies	Complies	Complies
Identification A	Retention time corresponds to standard	Complies	Complies	Complies
Identification B	UV spectrum corresponds to reference sample	Complies	Complies	Complies
Assay	95.0-105.0%	98.2%	96.6%	99.2%
Related	TBS1 RC4 ≤0.2%	BRT	BRT	BRT
Compounds	TBS1 RC5 ≤0.5%	0.2%	0.2%	0.2%
	Each individual unknown imp. ≤0.2%	BRT	BRT	BRT
	Total imp. ≤1.0%	0.2%	0.2%	0.2%

TABLE 3.2.P.5.4-2-continued

Batch Analysis - TBS-2 Bulk Gel, Batches IMP1 1005, IMP1 1006 and IMP1 1007				
Test Parameter	Acceptance Criteria	IMP1 1005	IMP1 1006	IMP1 1007
Viscosity	Report results	13,820 mPas	12,700 mPas	13,000 mPas
Water	Report results	0.2%	0.2%	0.2%
Residual	The product complies with USP<467> requirements	Complies	Complies	Complies

TBS1 RC4 - 17 β -hydroxyandrost-4,6-dien-3-one (Delta-6-testosterone);
 EP Impurity I TBS1 RC5 - 17 β -hydroxyandrost-4-en-3-one (Epitestosterone);
 EP Impurity C
 BRT—below reporting threshold

TABLE 3.2.P.5.4-3

Batch Analysis - TBS-2 Finished Product Filled in Multiple Dose Dispensers Batches IMP1 1008, IMP1 1009 and IMP1 1010				
Method	Specification	IMP11008	IMP11009	IMP11010
Appearance	White Albion barrel and cap filled with slightly yellow gel	Complies	Complies	Complies
Identification A	Retention time corresponds to standard	Complies	Complies	Complies
Identification B	UV spectrum corresponds to reference sample	Complies	Complies	Complies
Delivered Dose Uniformity	Beginning			
	Mean	98.5%	97.2%	98.2%
	Min	94.7%	95.5%	93.7%
	Max	100.5%	98.6%	100.4%
	% RSD	1.7%	1.1%	2.1%
	End			
Related Compounds	Mean	98.6%	97.3%	98.1%
	Min	97.3%	95.3%	96.1%
	Max	100.5%	101.5%	101.2%
	% RSD	1.0%	1.7%	1.7%
	TBS1 RC4 $\leq$ 0.2%	BRT	BRT	BRT
	TBS1 RC5 $\leq$ 0.5%	0.2%	0.2%	0.2%
Residual Solvents	Max individual unknown imp. $\leq$ 0.2%	BRT	BRT	BRT
	Total imp. $\leq$ 1.0%	0.2%	0.2%	0.2%
	The product complies with USP<467> requirements	Complies	Complies	Complies
Microbial Limits	TAMC $\leq$ 10 ² cfu/g	<10 ² cfu/g	<10 ² cfu/g	<10 ² cfu/g
	TYMC $\leq$ 10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
	<i>S. aureus</i> - must be absent in 1 g	0/g	0/g	0/g
	<i>P. aeruginosa</i> - must be absent in 1 g	0/g	0/g	0/g

TBS1 RC4 - 17 β -hydroxyandrost-4,6-dien-3-one (Delta-6-testosterone);
 EP Impurity I TBS1 RC5 - 17 β -hydroxyandrost-4-en-3-one (Epitestosterone);
 EP Impurity C
 TAMC—total aerobic microbial count;
 TYMC—total combined yeasts/mould count
 BRT—below reporting threshold

[1141] The only change made to the previous specification is the additional test for Delivered Dose Uniformity as per USP, to verify the performance of the dispenser. The following is a description of this test in the specification with a discussion concerning their suitability for intended use and a justification for the acceptable criterion.

[1142] The delivered dose of the gel discharged from the nasal actuator is analysed for the active content from a sample at the beginning and at the end of an individual multiple dose dispenser. Each dispenser is primed by actuating the pump 10 times. The drug content of the first dose, i.e. actuations #11 and #12 and the final dose (based on label claim of 10 doses,

i.e. 20 actuations), i.e. actuation #29 and #30 are tested using an in house HPLC method to determine the amount of active delivered from the nasal actuator, which is expressed as a percentage of the label claim.

[1143] The delivered dose uniformity test demonstrates the uniformity of the dose per actuation, and per total dose (2 actuations) and is consistent with the label claim, discharged from the nasal actuator.

[1144] The acceptance criteria proposed are based on USP <601> for Delivered Dose Uniformity and Guidance for Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products—Chemistry, Manufacturing and Controls Documentation:

[1145] The amount of active per determination is not outside of 80 to 120% of the LC for more than 2 of 20 determinations from 10 containers; none of the determinations is outside of 75 to 125% of the LC and the mean for each of the beginning and end determinations are not outside of 85 to 115% of LC.

[1146] TBS-2 is packaged in an Albion 15 ml barrel composed of polypropylene with a VP39/140H pump and cap. The barrel is filled with gel under vacuum using an Airless systems Laboratory filling unit (Airless systems, RD 149 Charleval 27380, France) to effectively expel 125 mg of gel.

[1147] The target fill weight for the Albion 15 ml barrel is 10.0 $\pm$ 1.0 g.

[1148] The quality control measures are proposed for the packaging components. Material information on the Albion 15 ml Barrel with VP 39/140H Pump and Cap is provided in Table 3.2.P.7-1. In addition to the supplier release CoA, the container closure system is retested upon receipt to conform to the specifications summarized in Table 3.2.P.7-2 The CoA for the Albion 15 ml Barrel Lot 10318UV12101 is provided in Appendix 5. A Figure of the 15 ml Albion Bottle and the PMP VP 39/140H 15 Albion+Digital Actuator+Cap 15 ml Albion Rounded is provided at FIG. 39.

TABLE 3.2.P.7-1

Albion 15 ml Barrel with VP 39/140H Pump and Cap - Material Information	
Component	Material
Barrel	Polypropylene: PPR 7220 White colorant: PP 00121522
Piston	Polyethylene PEHD PURELL GC 7260
Base	Polypropylene: PPR 7220 White colourant: PP 00121522
Insert	Polypropylene: PPH 7060
Pen actuator	Polypropylene: PPC 11712 White colourant: PP0012 1522
Snap on	Polypropylene: PPH 5060 White colourant: PP0012 1522
Neck gasket	Thermoplastic: F217-5 906
Stem gasket	Elastomer: 522C

TABLE 3.2.P.7-1-continued

Albion 15 ml Barrel with VP 39/140H Pump and Cap - Material Information	
Component	Material
Pump	
Body	PBT VALOX HX312C-1H1001
Ball support	HD Polyethylene: Purell GB7250
Ball	STAINLESS STEEL AISI 304L
Spring cap	POM HOSTAFORM C27021
Return spring	STAINLESS STEEL 1.43 10
Piston	HD Polyethylene: HOSTALEN GF4750
	SILBIONE DM300 GMP
Stem	POM DELRIN 900PNC 10
Pre compression spring	STAINLESS STEEL 1.43 10
Turret	Polypropylene: PPH5060
	White colourant: 85276

TABLE 3.2.P.7-2

Albion 15 ml Barrel with VP 39/140 Pump and Cap - Specifications	
Test Parameter	Acceptance Criteria
Appearance	Complies to current standard
Commodity no. of supplier	153963PH (SEB 15 Albion PP BC/4625 FDP BC/4625)
Colour - white	Must comply
Design	Complies technical drawing no. 01484 Index A (14.06.2007)
Total height	68.60 mm-69.70 mm
Outer diameter of bottle	24.80 mm-25.50 mm
Outer diameter of mouth	16.30 mm-16.45 mm
Compatibility with actuator	Must comply
Material type barrel - CoA	Polypropylene: PPR 7220, Colourant: white PP00121522
Material type piston - CoA	Polyethylene: HDPE Purell GC 7260
Material type bottom - CoA	Polypropylene: PR 7220, Colourant: white PP00121522
Identity - IR-spectrum of barrel	Must comply to reference spectrum
Packaging	Must comply
Attributive testing	AQL must comply
Certificate of Conformity of supplier	Must be given and comply

[1149] Three clinical batches bulk batches of TBS-2 (IMP1 1005, IMP1 1006 and IMP1 1007) were manufactured in a GMP facility. Each batch was then filled in the multiple dose dispenser as summarized in Table 3.2.P.8.1.

TABLE 3.2.P.8.1

Bulk Gel and Finished Product Batches			
Concentration	0.24%	0.48%	0.72%
Bulk Batch No.	IMP11005	IMP11006	IMP11007

TABLE 3.2.P.8.1-continued

Bulk Gel and Finished Product Batches			
Finished Product Batch No.	IMP11008	IMP11009	IMP11010

[1150] The bulk gel will stored under real time conditions $25\pm 2^{\circ}\text{C}.$, $60\pm 5\%$ RH and will be tested at the time intervals presented in Table 3.2.P.8.3.-2.

TABLE 3.2.P.8.3-2

Stability Schedule for TBS-2 Batches IMP1 1005, IMP1 1006 and IMP1 1007				
Storage Conditions ($^{\circ}\text{C}.$, % RH)	Strength	Batch Number	Product Type	Completed Test Intervals (Outstanding Test Intervals)
$25 \pm 2^{\circ}\text{C}.$, $60 \pm 5\%$ RH	0.24%	IMP11005	Bulk gel	0, (1 m, 2 m, 3 m, 6 m, 9 m, 12 m, 18 m,
$25 \pm 2^{\circ}\text{C}.$, $60 \pm 5\%$ RH	0.48%	IMP11006	Bulk gel	0, (1 m, 2 m, 3 m, 6 m, 9 m, 12 m, 18 m, 24 m, 36 m)
$25 \pm 2^{\circ}\text{C}.$, $60 \pm 5\%$ RH	0.72%	IMP11007	Bulk gel	0, (1 m, 2 m, 3 m, 6 m, 9 m, 12 m, 18 m, 24 m, 36 m)

TABLE 3.2.P.8.1-3

Stability Study Test Parameters of TBS-2 Bulk Gel and Corresponding Acceptance Criteria		
Test Parameter	Method/Reference	Acceptance Criteria
Appearance	STM.GEN.001	Slightly yellow gel
Identification A	STM.TBS2.001	Retention time and spectrum corresponds to standard
Assay	STM.TBS2.001	95.0-105.0%
Related Compounds	STM.TBS2.002	TBS1 RC4 $\delta 0.2\%$ TBS1 RC5 $\delta 0.5\%$ Individual unknown impurity $\delta 0.2\%$
Viscosity	USP <911>	Total impurities $\delta 1.0\%$
Water	USP <921> Method I	Report results
TBS1 RC4 - 17 β -hydroxyandrosta-4,6-dien-3-one (Delta-6-testosterone); EP Impurity I TBS1 RC5 - 17-hydroxyandrosta-4-en-3-one (Epitestosterone); EP Impurity C		

[1151] The multiple dose dispensers are stored under real time storage ($25\pm 2^{\circ}\text{C}.$, $60\pm 5\%$ RH), intermediate time storage ($30\pm 2^{\circ}\text{C}.$, $65\pm 5\%$ RH) and accelerated storage conditions ($40\pm 2^{\circ}\text{C}.$, $75\pm 5\%$ RH) and tested to the schedule presented in Table 3.2.P.8.1-4. Ten (10) dispensers will be analyzed at each time point.

TABLE 3.2.P.8.1-4

Stability Schedule COMPLEO Finished Product Filled in Multiple Dose Dispenser				
Storage Conditions ($^{\circ}\text{C}.$, % RH)	Batch Strength	Batch Number	Product Type	Completed Test Intervals (Outstanding Test Intervals)
$25 \pm 2^{\circ}\text{C}.$, $60 \pm 5\%$ RH	0.24%	IMP1 1008	Finished product in multiple dose dispenser	0 m, (3 m, 6 m, 9 m, 12 m, 18 m, 24 m, 36 m)

TABLE 3.2.P.8.1-4-continued

Stability Schedule COMPLEO Finished Product Filled in Multiple Dose Dispenser				
Storage Conditions (° C., % RH)	Batch Strength	Batch Number	Product Type	Completed Test Intervals (Outstanding Test Intervals)
30 ± 2° C., 65 ± 5% RH	0.24%	IMP1 1008	Finished product in multiple dose dispenser	0 m, (3 m, 6 m, 9 m, 12 m,)
40 ± 2° C., 75 ± 5% RH	0.24%	IMP1 1008	Finished product in multiple dose dispenser	0 m, (1 m, 2 m, 3 m, 6 m)
25 ± 2° C., 60 ± 5% RH	0.48%	IMP1 1009	Finished product in multiple dose dispenser	0 m, (3 m, 6 m, 9 m, 12 m, 18 m, 24 m, 36 m)
30 ± 2° C., 65 ± 5% RH	0.48%	IMP1 1009	Finished product in multiple dose dispenser	0 m, (3 m, 6 m, 9 m, 12 m)
40 ± 2° C., 75 ± 5% RH	0.48%	IMP1 1009	Finished product in multiple dose dispenser	0 m, (1 m, 2 m, 3 m, 6 m)
25 ± 2° C., 60 ± 5% RH	0.72%	IMP1 1010	Finished product in multiple dose dispenser	0 m, (3 m, 6 m, 9 m, 12 m, 18 m, 24 m, 36 m)
30 ± 2° C., 65 ± 5% RH	0.72%	IMP1 1010	Finished product in multiple dose dispenser	0 m, (3 m, 6 m, 9 m, 12 m)
40 ± 2° C., 75 ± 5% RH	0.72%	IMP1 1010	Finished product in multiple dose dispenser	0 m, (1 m, 2 m, 3 m, 6 m)

[1152] The attributes used to confirm the quality of TBS-2 finished product and corresponding acceptance criteria are listed in Table 3.2.P.8.1-5.

TABLE 3.2.P.8.1-5

Stability Study Test Parameters of TBS-2 Finished Product and Corresponding Acceptance Criteria		
Test Parameter	Method/Reference	Acceptance Criteria
Appearance	STM.GEN.001	White barrel and cap, filled with slightly yellow gel
Identification A	STM.TBS2.001	Retention time and spectrum corresponds to standard
Delivered Dose Uniformity	STM.TBS2.003	Meets USP <601> Mean (beginning doses & end doses) Min: Max: % RSD:
Related Compounds	STM.TBS2.002	TBS1 RC4 δ 0.2% TBS1 RC5 δ 0.5% Single unknown impurity δ 0.2%
Microbial Limits	USP<61>& <62>	Total impurities δ 1.0% Total aerobic microbial count <10 ² cfu/g Total combined yeasts/mould count <10 cfu/g

TABLE 3.2.P.8.1-5-continued

Stability Study Test Parameters of TBS-2 Finished Product and Corresponding Acceptance Criteria		
Test Parameter	Method/Reference	Acceptance Criteria
		<i>P. aeruginosa</i> 0/g <i>S. aureus</i> 0/g

[1153] The results from the stability studies will be evaluated for any trends and a shelf life proposed after data has been generated. Room temperature, intermediate and accelerated stability studies are ongoing and updated data are available upon request.

[1154] TBS-2 bulk and finished product will be placed on a stability program as described in section 3.2.P.8.1. Stability updates will be provided as available.

[1155] A 4.5% testosterone gel in accordance with this invention is packaged in the identical container closure system, the Albion 15 ml barrel composed of polypropylene with a VP39/140H pump and cap. The qualitative formulation is the same for the 4.5% gel as for the 0.24% 0.48% and 0.72% gels, only the proportion of castor oil and testosterone are different. Tables 3.2.P.8.3-1 and 3.2.P.8.3-2 provides supportive data for the stability of testosterone gel packaged in the Albion 15 ml barrel composed of polypropylene with a VP39/140H pump and cap. This supportive data indicates that the gel packaged in the dispenser provides adequate protection for potency and purity and are within acceptance criteria.

TABLE 3.2.P.8.3-1

Stability Data 4.5% Testosterone Gel Batch 1969 in Multiple Dose Dispensers, (25 ± 2° C., 60 ± 5% R.H., upright)						
Test	Specification	Time 0	3 months	6 months	9 months	12 months
Appearance	White barrel and cap filled with slightly	Complies	Complies			
Identification	Retention time of peak in the sample corresponds to standard	Complies	Complies			
Delivered Dose	Beginning and End must comply to USP<601>	Complies	Complies			
Uniformity	Beginning Mean	100.5%	100.2%			
	Min	96.0%	97.9%			
	Max	103.6%	102.1%			
	% RSD	2.1%	1.5%			
	End Mean	102.1%	100.1%			
	Min	99.9%	98.9%			
	Max	104.3%	102.7%			
	% RSD	1.3%	1.1%			
Related Compounds	TBS1 RC4 ≤ 0.2 ~	BRT	BRT			
	TBS1 RC5 ≤ 0.5%	0.3%	0.3%			
	Max individual	BRT	BRT			
	unknown imp. ≤ 0.2%					
	Total imp. ≤ 1.0%	0.3%	0.3%			
Microbial Limits	TAMC < 10 ² cfu/g	<10 cfu/g	—			
	TYMC < 10 cfu/g	<10 cfu/g	—			
	<i>S. aureus</i> -0/g	0/g	—			
	<i>P. aeruginosa</i> -0/g	0/g	—			

BRT—below reporting threshold

TABLE 3.2.P.8.3-2

Stability Data 4.5% Testosterone Gel Batch 1969 in Multiple Dose Dispensers, (40 ± 2° C., 75 ± 5% R.H., upright)						
Test	Specification	Time 0	1 months	2 months	3 months	6 months
Appearance	White barrel and cap filled with slightly	Complies	Complies	Complies	Complies	
Identification	Retention time of peak in the sample corresponds to	Complies	Complies	Complies	Complies	
Delivered Dose	Beginning and End must comply to USP<601>	Complies	Complies	Complies	Complies	
Uniformity	Beginning Mean	100.5%	105.0%	102.3%	100.6%	
	Min	96.0%	103.4%	100.5%	99.2%	
	Max	103.6%	106.2%	104.7%	101.4%	
	% RSD	2.1%	0.8%	1.5%	0.6%	
	End Mean	102.1%	104.6%	102.6%	100.3%	
	Min	99.9%	103.5%	100.5%	99.3%	
	Max	104.3%	107.0%	104.5%	101.4%	
	% RSD	1.3%	1.0%	1.1%	0.6%	
Related Compounds	TBS1 RC4 ≤ 0.2	BRT	BRT	BRT	BRT	
	TBS1 RC5 ≤ 0.5%	0.3%	0.3%	0.3%	0.3%	
	Max individual unknown imp. ≤ 0.2%	BRT	0.1%	0.1%	0.1%	
	Total imp. ≤ 1.0%	0.3%	0.4%	0.4%	0.4%	
Microbial Limits	TAMC < 10 ² cfu/g	<10 cfu/g	—	—	—	
	TYMC < 10	<10 cfu/g	—	—	—	
	<i>S. aureus</i> -0/g	0/g	—	—	—	
	<i>P. aeruginosa</i> -0/g	0/g	—	—	—	

BRT—below reporting threshold

14. Discussion and Overall Conclusions

A. Summary of Subjects

[1156] This was a phase 1, single-center, randomized, open-label parallel-group study of TBS-2 in 3 cohorts of subjects (Cohorts 1, 2, and 3) in Period 1 (single-dose) and a multiple-dose cohort in Period 2. A total of 24 healthy women received intranasal 600 µg, 1200 µg, or 1800 µg TBS-2 to evaluate the safety, tolerability, and PK of TBS-2.

B. Pharmacokinetic Conclusions

[1157] Results from the study indicate that the free testosterone concentrations and AUC_{0-8} , AUC_{0-24} , and $AUC_{0-\infty}$ parameters are dose proportional. While the total testosterone concentrations and parameters are clearly reflective of the dose, the strongest dose proportionality relationship is with the free testosterone. Dose proportionality is tested both by linear regression and by pair-wise dose comparison of parameters and both tests are equivocal in that free testosterone concentrations were dose proportional. Other analyte concentrations are less changed with the dose in the order of dihydrotestosterone with a minor change to estradiol with less and finally SHBG with the least change.

[1158] Additionally equivalence between the single- and multi-dose profiles is not rejected for either of the free testosterone AUC_{0-8} and AUC_{0-24} parameters suggesting little if any accumulation of free testosterone following multiple dosing. However, this result is limited to the 3 subjects who received 1200 µg on each profile. Single-dose parameter calculations are performed on uncorrected concentrations of free testosterone, total testosterone and dihydrotestosterone for comparison to multiple-dose parameters. The pharmacokinetic analysis is based on 5 endogenous analytes that have reported normal ranges as well as baseline ranges. The following table presents the normal ranges, the baseline ranges observed and a break-down of the baseline ranges by dose cohort.

Analyte		Normal Range	Pooled Baseline	Dose Cohort		
				600 (µg)	1200 (µg)	1800 (µg)
Free Testosterone (ng/dL)	Min	0.166	0.10	0.10	0.13	0.15
	Max	1.33	1.44	1.44	0.53	0.45
Total Testosterone (ng/dL)	Min	6.00	7.41	8.00	7.41	12.70
	Max	86.00	58.00	58.00	41.10	43.90
Dihydrotestosterone (ng/dL)	Min	4.00	5.02	5.29	5.04	5.02
	Max	22.00	32.60	22.10	25.30	32.60
Estradiol (pg/mL)	Min	20.00	17.00	20.90	17.00	17.70
	Max	241.00	122.00	122.00	119.00	97.40
SHBG (nmol/L)	Min	18.00	15.30	15.30	16.00	25.20
	Max	114.00	137.00	102.00	86.70	137.00

[1159] These data comparisons of the normal ranges with the pooled baseline ranges indicate that the pooled baseline ranges fall outside the normal ranges for free testosterone (min and max), dihydrotestosterone (max), estradiol (min) and SHBG (min and max).

C. Safety Conclusions

[1160] There have been no deaths, SAEs, or AEs leading to discontinuation during the study. Most TEAEs are in the

system organ classes of general disorders (nasal related events) and administration site conditions and respiratory, thoracic, and mediastinal disorders. This is not unexpected given the venipuncture for PK blood sample draws and the intranasal study drug administration. Most TEAEs are mild in severity and are unlikely or not related to study medication.

[1161] TBS-2 is believed to be safe and well tolerated with respect to AEs and vital signs.

[1162] The disclosures of the patents, patent documents, articles, abstracts and other publications cited herein are incorporated herein by reference in their entireties as if each were individually incorporated. In case of conflict, the present specification, including definitions, shall control. Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. Illustrative embodiments and examples are provided as examples only and are not intended to limit the scope of the present invention. The scope of the invention is limited only by the claims set forth as follows.

Having described our invention, we claim:

1. A testosterone gel formulation for nasal administration, said testosterone gel formulation comprising:

- about 0.15% testosterone by weight of said gel formulation; and
- a pharmaceutically acceptable vehicle.

2. The testosterone gel formulation of claim 1, wherein said gel formula further comprises a solvent, a wetting agent, and a viscosity increasing agent.

3. The testosterone gel formulation of claim 2, wherein said solvent is castor oil.

4. The testosterone gel formulation of claim 2, wherein said wetting agent is an oleoyl polyoxylglyceride.

5. The testosterone gel formulation of claim 2, wherein said viscosity increasing agent is colloidal silicon dioxide.

6. The testosterone gel formulation of any one of claims 1 or 2, wherein said gel formulation further comprises castor oil, oleoyl polyoxylglycerides and colloidal silicon dioxide.

7. The testosterone gel formulation of any one of claims 1-6, wherein said gel formulation is a bioequivalent formulation.

8. The testosterone gel formulation of any one of claims 1-6, wherein said gel formulation is a pharmaceutically equivalent formulation.

9. The testosterone gel formulation of any one of claims 1-6, wherein said gel formulation is a therapeutically equivalent formulation.

10. A packaged pharmaceutical comprising:

- (a) a testosterone gel formulation for nasal administration or a pharmaceutically acceptable salt or prodrug thereof, wherein said gel formulation comprises about 0.15% by weight; and
- (b) associated instructions for using said testosterone gel formulation to treat anorgasmia.

11. A packaged pharmaceutical comprising:

- (a) a testosterone gel formulation for nasal administration or a pharmaceutically acceptable salt or prodrug thereof, wherein said gel formulation comprises about 0.15% by weight; and
- (b) associated instructions for using said testosterone gel formulation to treat hypoactive sexual desire disorder (HSDD).

12. The packaged pharmaceutical of claim 10 or 11, wherein said testosterone is present as a pharmaceutical composition comprising a therapeutically effective amount of testosterone or a pharmaceutically acceptable salt or prodrug thereof and a pharmaceutically acceptable carrier.

13. The packaged pharmaceutical of claim 10 or 11, further comprising a step of identifying a subject in need of said pharmaceutical.

14. A method of treating anorgasmia comprising administering intranasally to each nostril of a subject said gel formulation of any one of claims 1-9 to deliver a therapeutically effective amount of testosterone to effectively treat anorgasmia.

15. A method of treating hypoactive sexual desire disorder (HSDD), comprising administering intranasally to each nostril of a subject said gel formulation of any one of claims 1-9 to deliver a therapeutically effective amount of testosterone to effectively treat HSDD.

16. An intranasal method of treating a subject diagnosed with anorgasmia with a testosterone gel formulation for nasal administration comprising about 0.15% testosterone by weight of said gel formulation, comprising: applying the testosterone gel formulation into each nostril of said subject at least once a day to deliver an effective amount of testosterone to treat the anorgasmia.

17. The method of claim 16, wherein said subject receives said testosterone gel intranasally twice daily.

18. An intranasal method of treating a subject diagnosed with HSDD with a testosterone gel formulation for nasal administration comprising about 0.15% testosterone by weight of said gel formulation, comprising: applying the testosterone gel formulation into each nostril of said subject at least once a day to deliver an effective amount of testosterone to treat the HSDD.

19. The method of claim 18, wherein said subject receives said testosterone gel intranasally twice daily.

20. A testosterone gel formulation for nasal administration, said testosterone gel formulation comprising:

- a. about 0.24% testosterone by weight of said gel formulation; and
- b. a pharmaceutically acceptable vehicle.

21. The testosterone gel formulation of claim 20, wherein said gel formula further comprises a solvent, a wetting agent, and a viscosity increasing agent.

22. The testosterone gel formulation of claim 21, wherein said solvent is castor oil.

23. The testosterone gel formulation of claim 22, wherein said wetting agent is an oleoyl polyoxylglyceride.

24. The testosterone gel formulation of claim 22, wherein said viscosity increasing agent is colloidal silicon dioxide.

25. The testosterone gel formulation of any one of claim 20 or 21, wherein said gel formulation further comprises castor oil, oleoyl polyoxylglycerides and colloidal silicon dioxide.

26. The testosterone gel formulation of any one of claims 20-25, wherein said gel formulation is a bioequivalent formulation.

27. The testosterone gel formulation of any one of claims 20-25, wherein said gel formulation is a pharmaceutically equivalent formulation.

28. The testosterone gel formulation of any one of claims 20-25, wherein said gel formulation is a therapeutically equivalent formulation.

29. A packaged pharmaceutical comprising:

- (a) a testosterone gel formulation for nasal administration or a pharmaceutically acceptable salt or prodrug thereof, wherein said gel formulation comprises about 0.24% by weight; and
- (b) associated instructions for using said testosterone gel formulation to treat anorgasmia.

30. A packaged pharmaceutical comprising:

- (a) a testosterone gel formulation for nasal administration or a pharmaceutically acceptable salt or prodrug thereof, wherein said gel formulation comprises about 0.24% by weight; and
- (b) associated instructions for using said testosterone gel formulation to treat hypoactive sexual desire disorder (HSDD).

31. The packaged pharmaceutical of claim 29 or 30, wherein said testosterone is present as a pharmaceutical composition comprising a therapeutically effective amount of testosterone or a pharmaceutically acceptable salt or prodrug thereof and a pharmaceutically acceptable carrier.

32. The packaged pharmaceutical of claim 29 or 30, further comprising a step of identifying a subject in need of said pharmaceutical.

33. A method of treating anorgasmia comprising administering intranasally to a subject said gel formulation of any one of claims 20-28 to deliver a therapeutically effective amount of testosterone to effectively treat HSDD.

34. A method of treating hypoactive sexual desire disorder (HSDD), comprising administering intranasally to each nostril of a subject said gel formulation of any one of claims 20-28 to deliver a therapeutically effective amount of testosterone to effectively treat HSDD.

35. An intranasal method of treating a subject diagnosed with anorgasmia with a testosterone gel formulation for nasal administration comprising about 0.24% testosterone by weight of said gel formulation, comprising: applying the testosterone gel formulation into each nostril of said subject at least once a day to deliver an effective amount of testosterone to treat the anorgasmia.

36. The method of claim 35, wherein said subject receives said testosterone gel intranasally twice daily.

37. An intranasal method of treating a subject diagnosed with HSDD with a testosterone gel formulation for nasal administration comprising about 0.24% testosterone by weight of said gel formulation, comprising: applying the testosterone gel formulation into each nostril of said subject at least once a day to deliver an effective amount of testosterone to treat the HSDD.

38. The method of claim 37, wherein said subject receives said testosterone gel intranasally twice daily.