

Approval Date: September 30, 2003

**FREEDOM OF INFORMATION SUMMARY
ORIGINAL NEW ANIMAL DRUG APPLICATION**

NADA 141-222

Altrenogest

MATRIX

**For synchronization of estrus in sexually mature gilts that
have had at least one estrous cycle.**

Sponsored By:

**Intervet, Inc.
P.O. Box 318
405 State Street
Millsboro, DE 19966**

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FREEDOM OF INFORMATION SUMMARY

MATRIX for Swine

1. GENERAL INFORMATION:

- a. File Number: NADA 141-222
- b. Sponsor: Intervet, Inc.
P.O. Box 318
405 State Street
Millsboro, DE 19966
Drug Labeler Code: 057926
- c. Established Name: Altrenogest
- d. Proprietary Name: MATRIX
- e. Dosage Form: Oral
- f. How Supplied: Altrenogest is supplied in a 1000 mL bottle
- g. How Dispensed: OTC
- h. Amount of Active Ingredients: 0.22% (w/v) altrenogest (2.2 mg/mL)
- i. Route of Administration: Oral
- j. Species/Class: Swine, sexually mature gilts
- k. Recommended Dosage: 15 mg altrenogest per gilt per day for 14 consecutive days (equivalent to 6.8 mL MATRIX 0.22% solution per head per day)
- l. Pharmacological Category: Steroid hormone
- m. Indications: For synchronization of estrus in sexually mature gilts that have had at least one estrous cycle.

2. **EFFECTIVENESS:**

a. Dosage Characterization:

Studies Investigating the Appropriate Dose of Altrenogest

Studies investigating the minimal effective dose of altrenogest for synchronization of estrus were largely based on regimens from scientists in France, which used 20 mg/head/day for 18 days (reviewed by Webel and Day, 1982). In the United States, Kraeling et al. (1981) examined a wide range of doses in different production environments to determine an effective dose of altrenogest for synchronization of estrus. In that study, doses of 5, 10, 20, or 40 mg/head/day were fed to gilts for 18 days. All doses of altrenogest were judged to synchronize estrus. However, the general trend was for the interval from the end of treatment to estrus to increase as the dose of altrenogest increased. There was also an increased incidence of large unovulated or cystic follicles on the ovaries of gilts fed the 5 and 10 mg doses. To corroborate this, Redmer and Day (1981a) found an increased incidence of cystic follicles in gilts given 2.5 vs. 15 mg/head/day altrenogest. Thus, Kraeling et al. (1981) concluded that to effectively synchronize estrus without causing increased cystic follicles, a dose between 10 and 20 mg/head/day should be administered.

To further characterize the dose response, Redmer and Day (1981b) treated gilts with 0, 10, 12.5, 15, 17.5, or 20 mg/head/day of altrenogest for 18 days. Similar to the findings of Kraeling et al. (1981), the duration from treatment cessation to estrus increased with the dose of altrenogest. The incidence of cystic follicles was low and unrelated to treatment. From this and previous research, Redmer and Day (1981b) concluded that a dose of 15 mg/head/day was optimal for synchronization of estrus in gilts

Studies Investigating the Length of Time of Altrenogest Feeding

In addition to the daily dose, the length and timing of administration of altrenogest is an important consideration for its use in synchronization of estrus in gilts. Stevenson and Davis (1982) fed 160 crossbred gilts 15 mg of altrenogest per day for 14 or 18 days beginning at diestrus (days +3 to +20 relative to estrus) or near estrus (days -1 to +2 relative to estrus). Day of estrus was day 0. There were no differences in the interval from withdrawal of treatment to estrus, farrowing rate, or numbers of pigs born alive between the gilts fed altrenogest for 14 or 18 days. In contrast, gilts near estrus when altrenogest feeding began exhibited a synchronous estrus, but the interval from withdrawal to onset of estrus was longer when compared with gilts whose treatment began during diestrus (5.6 vs. 5.2 days). These authors concluded that the 14-day treatment of gilts with 15 mg of altrenogest daily resulted in a synchronized, fertile estrus regardless of the stage of the estrous cycle when treatment was started.

Summary

Both the daily dose of altrenogest and the length of administration were important considerations when deciding the optimal dosage to test. Based on the above-cited results from published reports, the sponsor had adequate scientific rationale for selecting a daily dose of 15 mg/gilt for 14 days to test in their effectiveness study.

b. Substantial Evidence:

An adequate and well-controlled, multi-location effectiveness study was performed at the following four sites:

1. (a) Effectiveness Study Number: 97-0014

Investigator: Dan C. Ronning, M.S.
Colorado Animal Research Enterprises, Inc. (CARE)
Fort Collins, CO

(b) Effectiveness Study Number: 97-0015

Investigator: Eduardo Beltranena, I.A.Z., Ph.D.
Prairie Swine Centre, Inc.
Saskatoon, SK
Canada

(c) Effectiveness Study Number: 97-0018

Investigator: John J. Brennan, Ph.D.
Shur-Gain Agresearch
Burford, ONT
Canada

(d) Effectiveness Study Number: 97-0033

Investigator: Gary W. Davis, D.V.M., Ph.D.
Greenbriar Veterinary Services, Inc.
Delaware, OH

2. General Design

2.1 Study Type and Purpose

A multi-location effectiveness study was conducted to determine the effectiveness of altrenogest to synchronize estrus in sexually mature gilts. In addition, farrowing and weaning data were evaluated to determine the effects of altrenogest on the subsequent reproductive performance of study gilts.

2.2 Animals

Three hundred and twenty (320) crossbred gilts were used (80 animals at each of 4 sites). All animals selected for assignment to treatments exhibited estrus during a 28-day acclimation period and passed a veterinarian's examination for health, behavior, conformation, and appetite. At the end of the acclimation period, cyclic gilts were ranked from lightest to heaviest in terms of their body weight and grouped into pairs. Within each of these weight pairs, gilts were randomly assigned to treatment. Due to facility constraints, the study was conducted in two phases (40 animals in each phase) at each site.

2.3 Test Article Administration

Altrenogest was administered in liquid form as a "top dress" on approximately one-half of each gilt's daily feed. The altrenogest treatment (15 mg per head per day) was administered in 6.8 mL of a 0.22% solution. The placebo control consisted of 6.8 mL of the same liquid solution without altrenogest. After each gilt consumed the "top-dressed" portion of its feed, it received the remainder of its daily ration.

2.4 Experimental Procedures

At each site, gilts were treated daily during Study Days 1 to 14 or until they were observed in estrus, whichever occurred first. Gilts were observed twice daily for signs of estrus such as swollen vulva and immobilization response in presence of teaser boars. All gilts that exhibited estrus during Study Days 1 to 23 were bred via artificial insemination at approximately 10 to 14 hours and again 22 to 26 hours after first detection in estrus using pooled semen from boars within a given site. Gilts that failed to exhibit estrus during Study Days 1 to 23 were sacrificed and subjected to a thorough pathological examination. Gilts that returned to estrus after breeding were weighed and removed from the study. Pregnancy was diagnosed via ultrasound 28 to 45 days after first insemination. Gilts that exhibited no signs of estrus since breeding and were non-pregnant at pregnancy check were sacrificed and subjected to pathological examination. Pregnant gilts were transferred to farrowing crates approximately 110 days after first insemination and observed for signs of impending parturition. Date and duration (if possible) of parturition and the need for assisted farrowing (if applicable) were documented. During the postpartum period, gilts were observed for general health and incidence of metritis, mastitis, and agalactia. Pigs were weaned at 21 days postpartum and gilts were moved to available crates in the dry sow unit and were observed twice daily for estrus for 10 days following weaning. Gilts showing post-weaning estrus were weighed and removed from the study. Gilts that failed to exhibit post-weaning estrus were sacrificed and subjected to a pathological examination.

2.5 Variables Measured

Table 1 provides the pooled statistical analyses of response variables evaluated in the effectiveness study. Variables related to synchronization of estrus were used as the primary determinants of effectiveness. These variables include the proportion of all gilts in estrus during 19 to 21 days after the onset of treatment (i.e., 5 to 7 days after the end of treatment) and the proportion of all gilts in estrus during 18 to 23 days after the onset of treatment (i.e., 4 to 9 days after the end of treatment).

In addition to variables supporting effectiveness, several variables presented in Table 1 provide information regarding the animal safety of MATRIX (altrenogest) 0.22% Solution for Swine and supplement data from the Target Animal Safety Study presented in Section 3 of this FOI summary. These are related to reproductive safety of altrenogest and include variables such as conception rate, farrowing rate, post-weaning return to estrus, and litter characteristics.

During Phase 1 at the Colorado site, an outbreak of hemorrhagic enteritis/polyserositis caused by *Clostridium perfringens* and/or *E. coli* occurred. To control and treat this condition, many of the newborn pigs were treated with ceftiofur hydrochloride. However, due to differences in timing of parturition between treatment groups, significantly more pigs from altrenogest-treated gilts received ceftiofur hydrochloride as compared to the control group. Therefore, Phase 1 gilts (and their piglets) at this site were excluded from the statistical analyses of litter-related variables. Similarly, due to zeranolone contamination of the feed resulting in possible adverse reproductive effects during Phase 1 at the Ohio site, Phase 1 gilts at this site were excluded from the statistical analyses of reproductive and litter-related variables.

2.6 Statistical Analyses

Generalized linear mixed models (GLIMMIX macro of SAS) were used for data with binomial (proportions) and Poisson (counts) error distributions. For the continuous variables and those whose data were normally distributed, general linear mixed model procedures (PROC MIXED of SAS) were used. Timed events were analyzed by one of two procedures: 1) Survival analyses using proportional hazards regression (PHREG of SAS) if there were censored data; or 2) Log Rank Test (NPAR1WAY of SAS) if data were not censored.

3. Results

Table 1 provides the pooled statistical analyses of the response variables evaluated in the effectiveness study.

Administration of altrenogest at a dose of 15 mg/head/day for 14 days, suppressed expression of estrus during treatment. In addition, this treatment regimen successfully synchronized estrus, whether examining responses during study days 19-21 (days 5-7 post-treatment) or study days 18-23 (days 4-9 post-treatment). Thus, altrenogest is effective for synchronization of estrus in sexually mature gilts, the proposed claim for this product.

Reproductive performance of gilts given altrenogest was not compromised when compared to that of control gilts. There were no differences in gilt response variables such as conception rate, farrowing rate, post-weaning return to estrus, and gestation length. Relative to litter-related variables, no treatment differences were seen in litter size at birth, number of stillborn or mummified fetuses, number of pigs weaned, or other variables indicative of piglet viability. No treatment differences were seen in piglet birth and weaning weights, and growth rate.

Gilt body weight and body weight change within and across study periods did not differ between control and altrenogest treated gilts (data not shown).

Relative to animal safety, results from the effectiveness study indicate that altrenogest did not impair reproductive function when used to synchronize estrus in sexually mature gilts. Previously-published research that showed that underfeeding altrenogest may lead to increased incidence of cystic follicles (Kraeling et al., 1981; Redmer et al., 1981a, b), underscore the importance of assuring that each gilt gets the targeted daily dose of altrenogest.

4. Conclusions

Overall, results of the effectiveness study supports approval of the use of altrenogest to synchronize estrus in sexually mature gilts. Further, the results indicate that reproductive performance of treated gilts was not compromised.

Table 1. Effect of altrenogest on synchronization of estrus, gilt reproductive performance and piglet performance (pooled data from four sites)

Variable	Control	Altrenogest	P-value
Proportion of Gilts in Estrus During 1 to 14 Days After the Onset of Treatment	73/140 (52%)	5/138 (4%)	P < .001
Proportion of Gilts in Estrus During 19 to 21 Days After the Onset of Treatment	13/140 (9%)	106/138 (77%)	P < .001
Proportion of Gilts in Estrus During 18 to 23 Days After the Onset of Treatment	25/140 (18%)	117/138 (85%)	P < .001
Proportion of Gilts Bred During the First 23 Days After the Onset of Treatment	132/140 (94%)	126/138 (91%)	P = .336
Proportion of Bred Gilts that were Pregnant (Conception Rate)	115/132 (87%)	117/126 (93%)	P = .380
Proportion of Bred Gilts that Farrowed (Farrowing Rate)	97/132 (74%)	106/126 (84%)	P = .036
Proportion of Gilts that Exhibited an Estrus within 10 Days After Weaning a Litter	83/92 (90%)	100/103 (97%)	P = .177
Total Number of Pigs Born per Litter	10.8 (n = 83)	10.8 (n = 91)	P = .988
Number of Pigs Born Alive per Litter	9.5 (n = 82)	9.5 (n = 91)	P = .929
Number of Mummies per Litter	0.5 (n = 82)	0.4 (n = 91)	P = .939
Number of Stillbirths per Litter	0.9 (n = 82)	0.9 (n = 91)	P = .983
Number of Pigs Weighing Less than 1 kg at Birth per Litter	1.1 (n = 82)	1.2 (n = 91)	P = .598
Number of Pigs Born with Anatomical Abnormalities per Litter	0.1 (n = 82)	0.0 (n = 91)	P = .661

Table 1. Effect of altrenogest on synchronization of estrus, gilt reproductive performance and piglet performance (pooled data from four sites) (Continued)

Variable	Control	Altrenogest	P-value
Number of Pre-weaning Pig Deaths per Litter	1.2 (n = 82)	1.3 (n = 91)	P = .709
Number of Pigs Weaned per Litter	8.1 (n = 82)	8.2 (n = 90)	P = .723
Average Birth Weight (kg) of All Pigs Born	1.6 (n = 82)	1.6 (n = 92)	P = .961
Average Birth Weight (kg) of Pigs Born Alive	1.6 (n = 82)	1.6 (n = 94)	P = .863
Litter Birth Weight (kg) of All Pigs Born	16.2 (n = 82)	16.3 (n = 91)	P = .902
Litter Birth Weight (kg) of Pigs Born Alive	14.8 (n = 82)	15.0 (n = 91)	P = .997
Pre-weaning Mortality per Litter (No. of Pre-weaning Deaths/No. of Pigs Born Alive, %)	12.5 (n = 82)	13.6 (n = 91)	P = .182
Average Weaning Weight (kg) of Individual Pigs per Litter	6.6 (n = 78)	6.6 (n = 88)	P = .913
Litter Weaning Weight (kg)	53.4 (n = 78)	54.5 (n = 88)	P = .726
Average Daily Gain (kg/day) of Weaned Pigs per Litter	0.2 (n = 79)	0.2 (n = 90)	P = .791
Interval (Days) from Weaning to Estrus	4.9 (n = 89)	5.4 (n = 116)	P = .144
Gestation Length (Days)	115.6 (n = 103)	115.2 (n = 122)	P = .103

Literature Cited

- Kraeling, R.R., Dziuk, P.L., Pursel, V.G., Rampacek, G.B., and Webel, S.K. 1981. Synchronization of estrus in swine with allyl trenbolone (RU-2267). *J. Anim. Sci.* 52: 831-835.
- Redmer, D.A. and Day, B.N. 1981a. Ovarian activity and hormonal patterns in gilts fed allyl trenbolone. *J. Anim. Sci.* 53:1088-1094.
- Redmer, D.A., and Day, B.N. 1981b. Estrus and ovulation in gilts fed a synthetic progestogen. *Theriogenology* 16: 195-199.
- Stevenson, J.S., and Davis, D.L. 1982. Estrous synchronization and fertility in gilts after 14- or 18-day feeding of altrenogest beginning at estrus or diestrus. *J. Anim. Sci.* 55: 119-123.
- Webel, S.K., and Day, B.N. 1982. The control of ovulation. In: *Control of Pig Reproduction*. D.J.A. Cole and G.R. Foxcroft (eds.). Butterworth Scientific, London. pp. 197-210.

3. **TARGET ANIMAL SAFETY:**

Target Animal Safety Study Conducted by:

Dan. C. Ronning, Study Director
Colorado Animal Research Enterprises, Inc. (CARE)
Fort Collins, CO
Study Number: 96-0006

This study was performed in accordance with Good Laboratory Practice regulation 21 CFR Part 58 established by the Federal Food, Drug, and Cosmetic Act.

The objective of this study was to determine the effects of altrenogest when administered orally to cycling gilts. The treatments were 0 mg altrenogest per head per day for 14 days (Control), 15 mg altrenogest per head per day for 14 days (1X dose) and 150 mg altrenogest per head per day for 14 days (10X dose).

This study lasted approximately 204 days and encompassed the treatment, breeding, gestation, and lactation periods of breeding gilts. After a 43-day acclimation period, a group of 30 healthy cycling gilts were selected on the basis of health, conformation, behavior, body condition, appetite, and body weight and were randomly assigned to three treatment groups each comprised of 10 gilts. Test animals were fed their assigned treatment top-dressed on the feed once daily starting on Study Day 1 and continuing through Study Day 14. Estrus observations were performed twice daily from the beginning of the acclimation period through the end of the breeding period (Study Day 67). Gilts were inseminated at approximately 10 to 14 hours and again 22 to 26 hours after estrus was detected. Gilts were pregnancy tested by ultrasound at 45 to 48 days after insemination. Gilts that displayed estrus after the breeding period (Study Days 19 to 67) or were determined to be non-pregnant at pregnancy check were necropsied. Beginning on gestation day 108, pregnant gilts were observed twice daily for

signs of parturition and were moved to farrowing crates on gestation day 110. Following parturition, pigs were weaned at 21 days of age and were removed from the study. Pigs that died prior to 21 days of age were necropsied. Gilts that farrowed a litter were necropsied within five days of postpartum estrus but not later than 10 days following weaning.

Response variables included body weight and weight change, feed consumption, reproductive variables (days to post-treatment estrus, pregnancy rate to first insemination, fertilization rate, farrowing rate, gestation length, percentage of multiple matings, percentage of irregular returns to estrus, percentage of pregnant gilts that failed to farrow, days to postpartum estrus), litter variables (number of pigs born, number of pigs born alive, mummies or stillbirths, pre-weaning mortality rate, number of pigs weaned, birth weight, weaning weight, average daily gain from birth to weaning), clinical hematology variables (red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, white blood cell count, platelet count, differential blood cell count, prothrombin time, activated partial thromboplastin time), clinical chemistry variables (glucose, total protein, total non-esterified fatty acids, albumin, globulin, albumin to globulin ratio, serum urea nitrogen, creatinine, alkaline phosphatase, aspartate aminotransferase, amylase, gamma glutamyl transferase, sorbitol dehydrogenase, bilirubin, calcium, phosphorous, sodium, potassium, chloride), urine variables (specific gravity, pH, protein, glucose, ketones, bilirubin, blood), and organ weights.

Statistics:

The individual animal was the experimental unit for the statistical analyses. Analyses of treatment effects were considered statistically significant at the 10% level of significance. Treatment differences for repeated measures variables were subjected to analysis of variance or covariance (PROC MIXED or PROC GLM of SAS), using the measurement on Study Day -1 as the covariate for applicable analyses. Days to post-treatment estrus and days to postpartum estrus were analyzed using the Log Rank Test (PROC NPAR1WAY of SAS). Rate variables such as pregnancy rate were analyzed using Fisher's Exact Test (PROC FREQ option of SAS).

Results:

Table 2 provides the statistical analyses on the gilt reproductive performance and piglet performance variables evaluated in the study.

Table 2. Effect of altrenogest on gilt reproductive performance and piglet performance.

Variable	Treatment Group			P-value
	Control	1X Altrenogest	10X Altrenogest	
Days to Post-treatment Estrus (mean)	7.2 (n = 10)	5.6 (n = 10)	8.9 (n = 10)	P = .007
Pregnancy Rate to First Insemination (%)	90 (n = 10)	100 (n = 10)	70 (n = 10)	P = .142
Fertilization Rate (%)	82 (n = 10)	100 (n = 10)	69 (n = 10)	P = .142
Farrowing Rate (%)	90 (n=10)	100 (n=10)	80 (n=10)	P = 0.342
Gestation Length (mean days)	115.7 (n = 9)	115.9 (n = 10)	115.8 (n = 8)	P = .939
Percentage of Multiple Matings	10 (n = 10)	0 (n = 10)	30 (n = 10)	P = .142
Percentage of Pregnant Gilts that Failed to Farrow	0 (n = 9)	0 (n = 10)	11 (n = 9)	P = 0.348
Days to Postpartum Estrus (mean)	26.4 (n = 9)	25.6 (n = 10)	26.9 (n = 8)	P = .058
Number of Pigs Born per Litter (mean)	11.0 (n = 9)	9.0 (n = 10)	9.3 (n = 8)	P = .150
Number of Pigs Born Alive per Litter (mean)	9.7 (n = 9)	8.1 (n = 10)	9.0 (n = 8)	P = .522

Table 2. Effect of altrenogest on gilt reproductive performance and piglet performance. (Continued)

Variable	Treatment Group			P-value
	Control	1X Altrenogest	10X Altrenogest	
Number of Mummies per Litter (mean)	0.9 (n = 9)	0.0 (n = 10)	0.0 (n = 8)	P = .011
Number of Stillbirths per Litter (mean)	0.4 (n = 9)	0.9 (n = 10)	0.3 (n = 8)	P = .712
Pre-Weaning Mortality Rate (%)	3 (n = 9)	9 (n = 10)	12 (n = 8)	P = .247
Number of Pigs Weaned per Litter (mean)	9.3 (n = 9)	7.4 (n = 10)	7.9 (n = 8)	P = .544
Average Birth Weight per Pig (kg)	1.5 (n = 9)	1.4 (n = 10)	1.3 (n = 8)	P = .195
Average Weaning Weight per Pig (kg)	6.2 (n = 9)	6.2 (n = 10)	5.8 (n = 8)	P = .694
Average Daily Gain from Birth to Weaning (kg)	0.2 (n = 9)	0.2 (n = 10)	0.2 (n = 8)	P = .731

No differences among treatments were noted for pregnancy rate to first insemination, farrowing rate, gestation length, and postpartum return to estrus. Litter variables such as number of: pigs born, pigs born alive, pigs weaned, mummified fetuses, and stillbirths were similar among treatments. Pre-weaning mortality did not differ among treatments. Pig birth and weaning weights, and growth rate did not differ among treatments.

No differences among treatments were found in gilt body weights and weight changes within and across study periods (data not shown). Urinalysis and hematology variables, and organ weights also were unaffected by altrenogest treatment (data not shown).

Among gilts, gross lesions on necropsy were few in number, minor in nature and severity, and their occurrence was unrelated to treatment. Among piglets, there were no treatment-related differences in the frequency of gross lesions or cause of death.

No significant differences were observed among the three groups in clinical chemistry variables (data not shown) except chloride, sorbitol dehydrogenase (SDH), gamma glutamyl

transferase (γ GT), amylase, glucose, albumin, and albumin to globulin ratio (A/G ratio) when averaged over time. The mean chloride level for the 1X dose group was significantly lower than the 10X group. The mean SDH level and the mean glucose level for the 1X dose group were significantly higher than the 10X group and the control group. The mean γ GT level for the 1X dose group was significantly lower than the 10X group and the control group. The mean amylase level for the 10X group was significantly higher than the 1X group and the control group. The mean albumin level and mean A/G ratio for the control group were significantly lower than the other two treated groups. These isolated statistical differences among the three groups were small and values fell within normal ranges, and thus were not deemed to be biologically relevant.

Non-significant, treatment-related trends in stillbirth and pre-weaning mortality rate were noted. Since the size of the target animal safety study was too small to critically evaluate altrenogest's effects on the reproductive performance of gilts, variables related to reproductive safety were evaluated in the effectiveness study (see Section 2.b) where adequate animal numbers were available to make stronger conclusions as to altrenogest's possible effects on reproductive performance. In the effectiveness study, there were no negative effects of treatment on gilt reproductive performance or piglet performance. Litter size, number of stillbirths, and pre-weaning mortality rates were similar among treatment groups. Thus, possible concerns raised with results from the target animal safety study were adequately addressed in the effectiveness study, showing there were no differences in any variables related to piglet mortality and viability.

Conclusion:

Results from the target animal safety study indicate that there are no concerns with animal safety with respect to use of altrenogest in sexually mature gilts for synchronization of estrus. Results in the effectiveness study showed that altrenogest posed no reproductive safety concerns when used in gilts when used as directed in the label directions.

4. HUMAN SAFETY:

A. Toxicity:

Studies to assess the toxicity of altrenogest residues were conducted in a series of studies which included genetic toxicity (mutagenic potential), animal feeding, reproduction, and hormonal no-effect.

The pivotal studies that were conducted are listed as follows.

Mutagenic Potential Studies with Altrenogest

1. Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Allyl Trenbolone RU 2267
Huntingdon Research Centre, Report Number: RSL **351/781007**
December 18, 1978
2. Autoradiographic Assessment of DNA Repair in Mammalian Cells after Exposure to Altrenogest (RU 2267)
Huntingdon Research Centre, Report Number: RSL **610/83498**
June 29, 1983

3. Analysis of Metaphase Chromosomes Obtained from Bone Marrow of Rats Treated with Altrenogest
Huntingdon Research Centre, Report Number: RSL **609/83635**
September 14, 1983
4. An Assessment of the Mutagenic Potential of Altrenogest Using an *In Vitro* Mammalian Cell Test System
Huntingdon Research Centre, Report Number: RSL **611/83803**
October 13, 1983
5. The Hepatocyte Primary Culture/DNA Repair Assay on Altrenogest (RU-2267) Lot 2A-0548 Using Rat Hepatocytes in Culture
Barton Biotech Inc., New York, Report Number: BBI-**84-1**
May 18, 1984
6. An Assessment of the Mutagenic Potential of Altrenogest Using an *In Vitro* Mammalian Cell Test System
Huntingdon Research Centre, Report Number: RSL **611/637/8479**
February 10, 1984
7. An Assessment of the Mutagenic Potential of Altrenogest and 17 μ -Oestradiol in Mammalian Cells In Vitro using the Chinese Hamster Ovary/HGPRT Locus Assay
Huntingdon Research Centre, Report Number: RSL **671/85520**
May 2, 1985
8. Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Altrenogest
Huntingdon Research Centre, Report Number: RSL **672/85403**
June 7, 1985

Feeding Studies with Altrenogest

1. Toxicity of Rats in Dietary Administration Over 13 Weeks – Altrenogest
Huntingdon Research Centre, Report Number: RSL **588/83700**
February 10, 1984
2. 13-Week Oral Toxicity Study in Pigs - Altrenogest
Huntingdon Research Centre, Report Number: RSL **744/871485**
October 12, 1988
3. 1-Year Toxicity Study in Pigs - Altrenogest
Huntingdon Research Centre, Report Number: RSL **820/920468**
October 29, 1992

Reproduction Study with Altrenogest

1. Effect of Altrenogest on Reproductive Function of Multiple Generations in the Rat

Huntingdon Research Centre, Report Number: RSL **639/85167**
October 15, 1985

Hormonal No-Effect Study with Altrenogest

1. Determination of Hormonal No-Effect Dose Level for Altrenogest in the Rhesus Macaque
Oregon Regional Primate Research Center
April 22, 1983

A summary of each of these studies follows.

Mutagenic Potential Studies with Altrenogest

1. Report Number: RSL **351/781007**
 - a) Title: Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Allyl Trenbolone RU 2267
 - b) Report Date: December 18, 1978
 - c) Name(s) and Address(es) of Investigators:
David Hossack, Margaret Richold, Eryl Jones
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - d) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - e) Identity of Substance and Dosage Form Tested:
Technical Allyl Trenbolone (later renamed Altrenogest, Code No. RU 2267)
 - f) Species and Strain: Salmonella (5 strains)
 - g) Levels and Duration of Dosage: 10, 100, 1000, and 10,000 µg/plate
 - h) Route of Administration: Addition to soft agar plates
 - i) Parameter Studied: (Mean Number of Revertant Colonies per Plate):
 - j) Toxicities Observed: None
 - k) Conclusion(s): No evidence of mutagenic potential was observed in the bacterial test system at any dose level used.

2. Report Number: RSL **610/83498**

- a) Title: Autoradiographic Assessment of DNA Repair in Mammalian Cells after Exposure to Altrenogest (RU 2267)
- b) Report Date: June 29, 1983
- c) Name(s) and Address(es) of Investigators:
Jeffrey Allen, Raymond Proudlock
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- d) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- e) Identity of Substance and Dosage Form Tested: Altrenogest (Technical) (Code No. RU 2267)
- f) Species and Strain: *In Vitro* Study - Human Epithelioid (HeLa) Cells
- g) Levels and Duration of Dosage: 12.5 to 200 µg/mL for three hours.
- h) Route of Administration: Addition to cell suspension both with and without metabolic activation by S-9 mix.
- i) Parameter Studied: Reduction of DNA repair.
- j) Statistical Analysis: Two way analysis of variance.
- k) Conclusion(s): It is concluded that none of the doses of altrenogest used induce unscheduled DNA synthesis in HeLa cells as assessed by the ability of the compound to cause DNA repair, either in the presence or absence of S-9 mix.

3. Report Number: RSL **609/83635**

- a) Title: Analysis of Metaphase Chromosomes Obtained from Bone Marrow of Rats Treated with Altrenogest
- b) Report Date: September 14, 1983
- c) Name(s) and Address(es) of Investigators:
Jeffrey Allen, Raymond Proudlock, Paul Brooker, Anne Fox, Nigel Morgan
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- d) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- e) Identity of Substance and Dosage Form Tested: Altrenogest (Technical) (Code No. RU 2267)
- f) Species and Strain: Rats -- CD Rats of Sprague Dawley origin
- g) Number of Animals in Each Sex in Each Group: 5 males and 5 females
- h) Levels and Duration of Dosage: A single dose of 100 mg/kg with analysis at 6 and 30 hrs after treatment. Other groups were treated with 25 and 50 mg/kg/day for 4 days.
- i) Route of Administration: Intragastric intubation
- j) Parameter Studies: Incidence of cytogenetic damage in rats in bone marrow cell preparations
- k) Toxicities Observed: None
- l) Statistical Analysis: Kruskal-Wallis Test, Spearman's Correlation Test,

and Jonckheere's Test for Trend.

- m) Conclusion(s): Altrenogest did not show any evidence of mutagenic activity in this test procedure.

4. Report Number: RSL 611/83803

- a) Title: An Assessment of the Mutagenic Potential of Altrenogest Using an *In Vitro* Mammalian Cell Test System
- b) Starting Date: June 23, 1983
- c) Termination Date: August 30, 1983
- d) Report Date: October 13, 1983
- e) Name(s) and Address(es) of Investigators:
Margaret Richold, David Edgar, Sandra Ransome, Susan Banks
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- f) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical)
(Code No. RU 2267)
- h) Species and Strain: *In Vitro* Mammary Cell (Mouse Lymphoma) L5178Y Cells
- i) Levels and Duration of Dosage:
Concentrations of 22 to 90 µg/mL with and without metabolic activation,
with S-9 mix
- j) Route of Administration: Addition to suspension
- k) Parameter Studied: Detection and quantitation of forward mutation in mouse lymphoma cells from the heterozygous condition at the thymidine kinase locus to the thymidine kinase deficient genotype.
- l) Toxicities Observed: Altrenogest was found to be toxic to the cells both in the absence and presence of the S-9 mix.
- m) Statistical Analysis: Analysis of variance of the mutation frequency, with data transformed logarithmically.
- n) Conclusion(s): In the absence of S-9 mix, altrenogest shows no clear evidence of mutagenic potential. In the presence of S-9 mix, altrenogest shows evidence of weak mutagenic potential. This response is apparently non-specific, due to the fact that further testing (RSL 671/85520 and RLS 672/85403) done in presence or absence of exogenous metabolic activation did not show mutagenic effect.

5. Report Number: BBI-84-1

- a) Title: The Hepatocyte Primary Culture/DNA Repair Assay on Altrenogest (RU-2267) Lot 2A-0548 Using Rat Hepatocytes in Culture
- b) Starting Date: April 14, 1984
- c) Termination Date: May 14, 1984
- d) Report Date: May 18, 1984

- e) Name(s) and Address(es) of Investigators:
S. Ved Brat, D. Phil
Barton Biotech Inc.
Hawthorne, New York
 - f) Name and Address of Laboratory:
Barton Biotech Inc.
Hawthorne, New York
 - g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical) (RU 2267)
 - h) Species and Strain: Rats F-344 Hepatocytes
 - i) Levels and Duration of Dosage: 0.01 µg/mL to 2000 µg/mL solution added to the assay media
 - j) Route of Administration: Added to hepatocyte suspension
 - k) Parameter Studied: Cytotoxicity and Autoradiography grains/nucleus
 - l) Toxicities Observed: Cytotoxic at concentrations above 2 µg
 - m) No Observed Effect Level: 2 µg/mL
 - n) Statistical Analysis: Grain counts are determined with Artek Set in "Area Mode."
Positive is when the mean net nucleus grain count is 5 or more.
 - o) Conclusion(s): Altrenogest did not induce DNA repair when assayed in the rat hepatocyte primary cell culture/DNA repair assay at the highest non-toxic concentration of 2 µg/mL or at 5 additional lower concentrations.
6. Report Number: RSL **611/637/8479**
- a) Title: An Assessment of the Mutagenic Potential of Altrenogest Using an *In Vitro* Mammalian Cell Test System
 - b) Report Date: February 10, 1984
 - c) Name(s) and Address(es) of Investigators:
Margaret Richold, David Edgar, Sandra Ransome, Susan Banks, Helen Bosworth
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - d) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - e) Identity of Substance and Dosage Form Tested: Altrenogest (Technical) (Batch 2A-0645)
 - f) Species and Strain: Mammalian Mouse Lymphoma L5178Y Cells
 - g) Levels and Duration of Dosage: Concentration of 20 to 100 µg/mL with and without metabolic activation with S-9 Mix.
 - h) Route of Administration: Addition to suspension
 - i) Parameter Studied: Detection and quantitation of forward mutation in mouse lymphoma cells from the heterozygous condition at the thymidine kinase locus to the thymidine kinase deficient genotype.

- j) Toxicities Observed: Dose dependent on cytotoxicity in the cells over concentration ranges between 38 and 62 µg/mL.
- k) Statistical Analysis: Analysis of Variance of the mutation frequencies after data had been transformed logarithmically.
- l) Conclusion(s): In the absence of exogenous metabolic activation, there was no demonstration of mutagenic potential. In the presence of metabolic activation, highly variable increase in mutation frequency was stimulated. This response is apparently non-specific, due to the fact that further testing (RSL 671/85520 and RLS 672/85403) done in presence or absence of exogenous metabolic activation did not show mutagenic effect.

7. Report Number: RSL **671/85520**

- a) Title: An Assessment of the Mutagenic Potential of Altrenogest and 17µ-Oestradiol in Mammalian Cells *In Vitro* using the Chinese Hamster Ovary/HGPRT Locus Assay
- b) Starting Date: February 7, 1985
- c) Termination Date: April 10, 1985
- d) Report Date: May 2, 1985
- e) Name(s) and Address(es) of Investigators:
Margaret Richold, David Edgar, Sandra Ransome, Susan Banks, Helen Bosworth
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- f) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical)
- h) Species and Strain: Chinese Hamster Ovary (CHO) cells (*In Vitro*)
- i) Levels and Duration of Dosage: 0-40 µg/mL main test without metabolic activation; 0-50 µg/mL with metabolic activation - incubated for 7 days.
- j) Route of Administration: Administration to cell culture media
- k) Parameter Studied: Cytotoxicity and mutant frequency expressed as the number of mutant (TG) resistant colonies per 10⁶ viable cells
- l) Toxicities Observed: Cytotoxic concentrations above 30 µg/mL
- m) Statistical Analysis: Linear regression analysis.
- n) Conclusion(s): Altrenogest induced a concentration-dependent decrease in cell survival in the preliminary test and in the main test in the presence and absence of exogenous metabolic activation, but failed to stimulate any significant concentration-dependent increase in mutant frequency in any of the tests carried out. Altrenogest did not show any mutagenic potential in this mammalian test system.

8. Report Number: RSL **672/85403**

- a) Title: Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Altrenogest
- b) Starting Date: February 25, 1985
- c) Termination Date: March 2, 1985

- d) Report Date: June 7, 1985
- e) Name(s) and Address(es) of Investigators:
Jeffrey Allen, Paul Brooker, Kathryn McCaffrey
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- f) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical)
- h) Species and Strain: Chinese Hamster Ovary Tissue, *In Vitro* (CHO-K₁ cells of the BH₄ subclone)
- i) Levels and Duration of Dosage: 12.5 - 100 µg/mL in preliminary test; 5, 7.5, and 10 µg/mL main test without metabolic activation; 5, 25, and 50 µg/mL with metabolic activation - incubated for 20 hours.
- j) Route of Administration: Administration to cell culture media
- k) Parameter Studied: Cytotoxicity as determined by mitotic indices and metaphase analysis, number and type of chromosome aberrations.
- l) Toxicities Observed: EC₅₀ of 10 µg/mL without metabolic activation and 50 µg/mL with metabolic activation.
- m) Statistical Analysis: Fisher's Exact Test
- n) Conclusion(s): Altrenogest showed no clear evidence of clastogenic activity in this cytogenetic toxicity study using CHO cells cultured *in vitro*.

Feeding Studies with Altrenogest

1. Report Number: RSL **588/83700**
 - a) Title: Toxicity of Rats in Dietary Administration Over 13 Weeks
 - b) Starting Date: March 3, 1983
 - c) Termination Date: June 6, 1983
 - d) Report Date: February 10, 1984
 - e) Name(s) and Address(es) of Investigators:
Owen Green, Ralph Heywood, Alan Street, Chirukandath Gopinath
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - f) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical)
(Code No. RU 2267) (Batch No. 2A645 mixed into feed for dietary administration)
 - h) Species and Strain: Rats, CD of the Sprague Dawley Strain
 - i) Number of Animals in Each Sex in Each Group: 15 males and 15 females per test group

- j) Levels and Duration of Dosage: Treatment levels of control 1, 10, and 100 ppm continuously for 13 weeks.
- k) Route of Administration: Dietary
- l) Parameter Studied: Clinical signs, mortality, body weight, food consumption, water consumption, ophthalmoscopy, comprehensive hematology, comprehensive blood chemistry, urinalysis, post-necropsy, necropsy (organ weights), gross observation, and histopathological examination.
- m) Toxicities Observed: Lower white blood cells, neutrophil and lymphocyte counts for males treated with 100 ppm, small testes, prostate, and empty seminal vesicles for males treated with 100 ppm. Lower adrenal weights noted in males treated with 100 ppm. Lower ovary weights in females treated with 100 ppm. Reduced spermatogenesis at 100 ppm, reduction in colloid seen in prostate of males treated with 100 ppm with minimal reduction in colloid alone.
- n) No Observed Effect Level: A level of 10 ppm showed minimal toxicity, whereas, a level of 1 ppm was considered a no-effect level.
- o) Statistical Analysis: Food consumption, body weight, and water consumption data were assessed by a one-way analysis of variance and intergroup comparison made by using Student's t test based on the residual variance (i.e., using the method of Least Significant Differences).

The following sequence of statistical tests were used for any variable in which the relative frequency of the mode was less than 75% (i.e., the data did not consist predominantly of one particular value).

- Bartlett's test was applied to test for homogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a log transformation was tried to see if the heterogeneity was removed. A square root transformation was also tried if the log transformation was not satisfactory.
- If no significant heterogeneity was detected (or if a satisfactory transformation was found by the process described above), a (one-way) analysis of variance was carried out. If no significant transformation was found, the Kruskal-Wallis analysis of ranks was used.
- Analysis of variance was followed by Student's t test and Williams' test for a dose-related response. The Kruskal-Wallis analysis was followed by the non-parametric equivalents of the t test and William's test.

For those variables in which the relative frequency of the mode was 75% or more, the number of animals in each group with values different from the mode was analyzed using Fisher's Exact test and Mantel's test. Fisher's test was used to detect general differences between treatments, whereas Mantel's test was used specifically for detecting dose-related trends in the numbers of such animals.

- p) Conclusion(s): Altrenogest had an effect on the male reproductive organs and a reduction in white blood cells parameters in rats treated with a level of 100 ppm altrenogest in the diet. At 10 ppm altrenogest showed minimal toxicity. One (1) ppm was considered a no-effect level.

In the diet, 1 ppm was equivalent to 60 µg/kg body weight/day of male rats and 80 µg/kg body weight/day of female rats.

2. Report Number: RSL 744/871485

- a) Title: 13-Week Oral Toxicity Study in Pigs
- b) Starting Date: June 26, 1987
- c) Termination Date: October 2, 1987
- d) Report Date: October 12, 1988
- e) Name(s) and Address(es) of Investigators:
Nicholas Roberts, David Crook, David Cameron, Geoffrey Brown,
Chirukandath Gopinath, Cora Cherry
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- f) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical) (Code No. RU 2267) Lot No. 6J0831: (17 α allyl estratriene 4, 9, 11, 17 beta-ol-3one)
- h) Species and Strain: Porcine - Large white hybrid pigs
- i) Number of Animals in Each Sex in Each Group: 32 week old pigs. 4 males and 4 females per test group
- j) Levels and Duration of Dosage: 0, 4, 40, and 200 µg/kg/day for 13-weeks
- k) Route of Administration: Dietary
- l) Parameter Studied: Clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, fecal occult blood, macroscopic post-mortem examination, and histological examination.
- m) Toxicities Observed: Suppression of estrus in female pigs at 40 and 200 µg/kg. Decrease in testes, prostate, and epididymis weights in male pigs at 40 and 200 µg/kg. Decrease in ovarian weight in female pigs at 40 and 200 µg/kg. Testicular interstitial cell atrophy and reduced prostatic development in male pigs at 40 and 200 µg/kg. Suppression of ovarian cycle activity and reduced uterine development in female pigs at 40 and 200 µg/kg.
- n) No Observed Effect Level: 4 µg/kg/day
- o) Statistical Analysis: Organ weights, Williams' test for non-parametric equivalent. two-way analysis of variance with block and treatment as factors. Means were compared using Williams' test for a series of increasing dose levels. Hematology, biochemistry, and urinalysis, Williams' test for non-parametric equivalent.
- p) Conclusion(s): Following the treatment of sexually mature male and female pigs with altrenogest administered by incorporation in the feed to give dosages of 4, 40, or 200 µg/kg body weight per day, no adverse effects of treatment on clinical health, weight gain, food consumption, or clinical pathological parameters were observed.

At termination, no macroscopic abnormalities were seen which could be associated with experimental treatment. In male pigs in Groups 3 (40 µg/kg/day) and 4 (200 µg/kg/day), significant decreases in prostate, testis,

and epididymis weights were observed in comparison with controls, and mean seminal vesicle weights were reduced (though this difference was not significant). Female pigs in Groups 3 and 4 had significantly lower mean ovarian weights (and higher kidney weights) than controls. Organ weights for male and female pigs in Group 2 (4 µg/kg/day) were not significantly different from controls.

Histopathological findings showed dose-related effects in Groups 3 and 4 associated with the hormonal action of the test compound (testicular interstitial cell atrophy and reduced prostatic development in males, suppression of ovarian cyclic activity and associated uterine development in females).

No treatment-related histopathological effects were seen in male or female pigs in Group 2 (4 µg/kg/day).

3. Report Number: RSL 820/920468

- a) Title: 1-Year Toxicity Study in Pigs
- b) Starting Date: October 10, 1990
- c) Termination Date: January 29, 1992
- d) Report Date: October 29, 1992
- e) Name(s) and Address(es) of Investigators:
David Cameron, David Crook, Chirukandath Gopinath, Christine Parker,
William Gibson, John Offer
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- f) Name and Address of Laboratory:
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical)
(Code No. RU 2267) Lot No. 9V0886B
- h) Species and Strain: Porcine - Large white hybrid pigs (*Sus scrofa*)
- i) Number of Animals in Each Sex in Each Group: 8 males and 8 females per treatment group
- j) Levels and Duration of Dosage: 0, 0.5, 1.0, and 10 ppm in feed (dietary) equivalent to dose level of 0, 2, 4, and 40 µg/kg/day
- k) Route of Administration: Dietary
- l) Parameter Studied: Clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, necropsy gross, and histology.
- m) Toxicities Observed: Significant treatment-related reductions in the mean weight testes, epididymis, seminal vesicles, prostate, and uterus at 40 µg/kg/day. Increase in serum concentration of albumin and cholesterol at 40 µg/kg/day. Mean pituitary weight at 40 µg/kg/day was significantly increased. Increase in weight gains in female pigs at 4 and 40 µg/kg/day. Microscopic changes in males consisted of testicular interstitial cell reduction.
- n) No Observed Effect Level: The toxicological no-effect level in this study was determined to be 2 µg/kg/day.

- o) **Statistical Analysis:** All statistical analyses were carried out separately for males and females. The following sequence of statistical tests was used for clinical pathology and organ weight data.
- If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analyzed by appropriate methods. Otherwise:
 - Bartlett's test was applied to test for homogeneity of variance between treatments. Where significant heterogeneity (at the 1% level) was found, a logarithmic transformation was tried to determine if a more stable variance structure was obtainable.
 - If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and was not removed by a transformation, the Kruskal-Wallis analysis of ranks was used.
 - Except for pre-dose data, analysis of variance was followed by t test (reported to 5% and 1% levels) and/or Williams' test as appropriate to determine any significant differences; the Kruskal-Wallis analysis was followed by the non-parametric equivalents of the t test and Williams' test (Shirley's test).
- Where appropriate, analysis of covariance was used in place of analyses of variance. For organ weight data, the final body weight was used as a covariate in order to allow for differences in body weight which may influence the organ weights.
- p) **Conclusion(s):** The primary effects of altrenogest in this study were related to the hormonal effects of the substance. The dosage of 2 µg/kg/day was determined to be a general no-adverse effect level.

Reproduction Study with Altrenogest

1. Report Number: RSL **639/85167**
- a) Title: Effect of Altrenogest on Reproductive Function of Multiple Generations in the Rat
 - b) Starting Date: January 18, 1984
 - c) Termination Date: November 2, 1984
 - d) Report Date: October 15, 1985
 - e) Name(s) and Address(es) of Investigators:
James Edwards, Yvonne Reid, David John, W.A. Gibson, John Offer
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
 - f) Name and Address of Laboratory:
Huntingdon Research Centre

Huntingdon, Cambridgeshire, UK

- g) Identity of Substance and Dosage Form Tested: Altrenogest (Technical) (Code No. RU 2267) Batch No. 2A 0645
- h) Species and Strain: Rats, Crl: COBS CD (SD) BR strain
- i) Number of Animals in Each Sex in Each Group: 15 rats/sex/dose
- j) Levels and Duration of Dosage: 0, 0.4, 4, and 40 ppm – 13 weeks
- k) Route of Administration: Dietary
- l) Parameters Studied: Clinical signs, mortalities, food consumption, water consumption, body weight, macroscopic abnormalities, pregnancy rate, mating performance, gestation period, litter data, pre-weaning development, and necropsy. Malformations, anomalies, or variants in pups from F0 females, 2nd mate, day 20 of pregnancy.
- m) Toxicities Observed: Mating performance, pregnancy rate, and litter size in the 40 ppm group were lower than control group. At 40 ppm in the diet there were reduced weight of the testes, epididymis, and seminal vesicles/prostate in treated males and increased anogenital distance of female fetuses. The mean relative weight of the kidneys and adrenals were significantly lower and the liver was higher in the 40 ppm group.

At 4 ppm, there was marginally higher body weight gains for F0 and F1 treated males and reduced seminal vesicles/prostate weight of F0 males.

No evidence of teratological effects in pups.

- n) No Observed Effect Level: The no-effect level was 0.4 ppm in the diet. This was equivalent to 16 µg/kg body weight/day (under the conditions of this study).
 - o) Statistical Analysis: Performed routinely on litter data. Non parametric tests (i.e., Jonckheere and Kruskal-Wallis tests) were employed routinely for litter parameters as these values rarely follow a 'normal' distribution. Fisher's Exact test was also used where the incidence of tied values proved the Kruskal-Wallis test to be inappropriate.
- Fisher's Exact test was used for incidence values such as pregnancy rate and the duration of gestation.
 - Weekly body weight gains of adults were analyzed by analysis of variance in conjunction with Williams' test.
 - Organ weights were analyzed by analysis of variance adjusting for body weight at sacrifice as covariate provided there was found to be a significant relationship (F-test $P < 0.1$). Treatment means were compared with control values by the method of LSD's in conjunction with Williams' test.

- p) Conclusion(s): The effects of altrenogest were related to the hormonal activity of the substance. There were no teratological or embryotoxic effects. Following withdrawal of treatment at weaning, the reproductive performance of F1 animals was unaffected at all dietary concentrations.

Hormonal No-Effect Study with Altrenogest

1. Report Number: Un-numbered final report
 - a) Title: Determination of Hormonal No-Effect Dose Level for Altrenogest in the Rhesus Macaque
 - b) Starting Date: November 1, 1981
 - c) Termination Date: February 28, 1983
 - d) Report Date: April 22, 1983
 - e) Name(s) and Address(es) of Investigators:
David Hess, Ph.D.
Oregon Regional Primate Research Center
Beaverton, Oregon
 - f) Name and Address of Laboratory:
Oregon Regional Primate Research Center
Beaverton, Oregon
 - g) Identity of Substance and Dosage Form Tested:
Altrenogest (Technical) (Code No. RU 2267) Batch 28, Lot No. OE 0314
 - h) Species and Strain: Monkeys, mature Rhesus Macaques
 - i) Number of Animals in Each Sex in Each Group: 6 females in each group
 - j) Levels and Duration of Dosage: Dosages of 12, 24, or 48 µg/day (approximately 2, 4, or 8 µg/kg) for three menstrual cycles.
 - k) Route of Administration: Orally in formulated diet cube. Daily administration of a single 17 gram cube containing the appropriate dosage.
 - l) Parameter Studied: Menstrual cycle data and serum hormonal.
Estradiol-17β
Progesterone
Luteinizing Hormone
Follicle stimulating hormone
 - m) Toxicities Observed: Effects were those expected as related to the hormonal activity of the test substance.
 - n) No Observed Effect Level: The antigonadotropic hormonal no-effect dose level for altrenogest is 24 µg/day or approximately 4 µg/kg/day in this species.
 - o) Statistical Analysis: Means and standard errors of the mean for age, weight, dose, and menstrual cycle information for hormonal analysis means with 95% confidence intervals.
 - p) Conclusion(s): Partial or complete inhibition of menstrual cyclicity and the serum steroid and gonadotropin profiles associated with such cycles were observed in most of the animals at the highest dose tested, while the mid and low dose were without effect on any of the variables measured.

B. Safe Concentration of Total Residues:

1. No-Observed Effect Level (NOEL)

The safe concentration of total residue was determined from the lowest no-effect level in the most sensitive and appropriate species from the various toxicological studies conducted. The lowest NOEL used in calculation of safe concentration is that determined in the hormonal no-effect study in Rhesus monkeys, 4 µg/kg body weight/day.

2. Calculation of the Acceptable Daily Intake (ADI)

Calculations of acceptable daily intake were made based on a no-observed effect level of 4 µg/kg body weight/day. With an 100-fold safety margin, the Acceptable Daily Intake (ADI) is .04 µg/kg/day. This is equivalent to 2.4 µg/60 kg person/day.

3. Calculation of the Safe Concentrations

The following equation was used in calculation of safe concentrations (SC):

$$SC = ADI \times \frac{60 \text{ kg}}{\text{consumption factor (in kg/day)}}$$

$$SC (\text{Muscle}) = .04 \text{ µg/kg bw/day} \times \frac{60 \text{ kg}}{.3} = 8 \text{ ppb}$$

$$SC (\text{Liver}) = .04 \text{ µg/kg bw/day} \times \frac{60 \text{ kg}}{.1} = 24 \text{ ppb}$$

$$SC (\text{Kidney}) = .04 \text{ µg/kg bw/day} \times \frac{60 \text{ kg}}{.05} = 48 \text{ ppb}$$

$$SC (\text{Fat}) = .04 \text{ µg/kg bw/day} \times \frac{60 \text{ kg}}{.05} = 48 \text{ ppb}$$

Liver is the target tissue, thus the safe concentration in liver as calculated above is used for comparison with the liver residue data.

4. Threshold Assessment

The battery of genetic toxicity tests were conducted to establish mutagenic potential of the substance. Additional studies as described in the General Principles for Evaluating the Safety of Compounds Used in Food Producing Animals, Revised July 1994, were conducted as it relates to consideration of synthetic sex-steroids. Based on the results of these studies, it was determined that altrenogest could be evaluated under the General Food Safety Criteria.

C. Total Residue Depletion and Metabolism Studies:

The following studies were conducted to characterize and quantitate the residues of altrenogest:

Residue Studies of Altrenogest

1. Tissue Residues of Radioactivity After Oral Doses of [6, 7 - ³H] - Allyl Trenbolone to Pigs
Huntingdon Research Centre, Report Number: RSL **326/79531**
June 25, 1979
2. Concentrations of Radioactivity in the Tissues of Pigs After Repeated Oral Doses of ³H-Allyl Trenbolone
Huntingdon Research Centre, Report Number: RSL **407/81105**
March 27, 1981
3. An Investigation of the Nature of Radioactive Residues in the Livers of Pigs after Repeated Oral Administration of ³H-Allyl Trenbolone
Huntingdon Research Centre, Report Number: RSL **539/82914**
October 20, 1982
4. Tissue Distribution of Radioactivity and Patterns of Metabolites following Single and Multiple Doses of ³H-Altrenogest to Pigs
Huntingdon Research Centre, Report Number: RSL **616/84177**
June 13, 1985
5. Residue Depletion in Liver of Mature Gilts - Altrenogest
Huntingdon Research Centre, Report Number: RSL **769/89220**
May 3, 1989
6. Bioavailability of Altrenogest Residues in Pig Liver following Oral Administration to Rats
Huntingdon Research Centre, Report Number: RSL **726/861541**
November 26, 1986
7. An Evaluation of the Extractability of Radioactivity from the Livers of Pigs Treated with ³H-Altrenogest
Huntingdon Research Centre, Report Number: RSL **819/920064**
January 31, 1992
8. Tissue Residue Depletion Study Following Administration of REGU-MATE to Gilts
Bio Logic, Trial 98-030-P-3
July 26, 1999

A summary of each of the residue studies follows.

1. Report Number: RSL **326/79531**
 - a) Title: Tissue Residues of Radioactivity After Oral Doses of [6, 7 - ³H] - Allyl Trenbolone to Pigs
Huntingdon Research Centre
 - b) Report Date: June 25, 1979
 - c) Name(s) and Address(es) of Investigators:
David Hawkins, D. G. Cresswell, J.A. Savage, Elliott, David Ross,
D. M. Cameron

Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK

- d) Description of Animals:
Pigs, young adult females, large white breed. 2 animals in preliminary study and 7 animals in main study - Weights from 120 - 150 kg.
- e) Route of Drug Administration: Gastric intubation. [6,7-³H]altrenogest (specific activity=56 Ci/mmol)
- f) Time and Duration of Dosing:
Preliminary study - Single dose of approximately 20 mg as a solution, 20% aqueous.

Main study - Seven consecutive daily doses of 20 mg/animal.

Particular isotope used - tritiated allyl trenbolone (later renamed, altrenogest). Tritium replaced hydrogen on the 6 and 7 position.
- g) Withdrawals:
Preliminary Study - pig 1 (48 Hours), Pig 2 (120 Hours)

Main Study - 24 to 360 Hours
- h) Summary of Average Total Residue Concentration:

Table 3: Preliminary Study

Tissue	Animal #1 ppb	Animal #2 ppb
Liver	49	20
Kidney	22	7
Leg Muscle	2	1
Shoulder Muscle	2	1
Renal Fat	5	1
Subcutaneous Fat	6	1
Plasma	3	2

Table 4: Main Study

Tissue	Hours Withdrawal*						
	24	48	72	120	168	240	360
Liver	432	172	190	105	46	48	36
Kidney	122	96	77	39	24	22	12
Leg Muscle	11	20	10	8	4	3	4
Shoulder Muscle	10	13	13	8	4	3	3
Renal Fat	33	14	6	6	ND	ND	ND
Subcutaneous Fat	22	12	7	7	ND	ND	ND
Plasma	24	19	20	11	12	9	ND

* ND = Not Detected
Results are expressed as ppb

2. Report Number RSL 407/81105

- a) Title: Concentrations of Radioactivity in the Tissues of Pigs After Repeated Oral Doses of ³H-Allyl Trenbolone
Huntingdon Research Centre,
- b) Report Date: March 27, 1981
- c) Name(s) and Address(es) of Investigators:
D.R. Hawkins, R. Girkin, N.L. Roberts, D. M. Cameron
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- d) Description of Animals: Pigs, young adult females, large white breed. 12 animals, Weights 170 kg.
- e) Route of Drug Administration: Gastric intubation.
- f) Time and Duration of Dosing: Single dose for 18 days of approximately 20 mg as a solution, 20% aqueous.
Particular isotope used – 15 mg (30 mCi) tritiated allyl trenbolone (later renamed, altrenogest). Tritium replaced hydrogen on the 6 and 7 position.

g) Summary of Average Total Residue Concentration:

Table 5

Tissue	Day 15 ppb	Day 30 ppb	Day 60 ppb	Day 179 ppb
Liver	60.3	26.1	15.6	10.1
Kidney	15.7	5.7	1.9	- *
Leg Muscle	5.2	1.8	-	-
Shoulder Muscle	5.0	1.9	-	-
Renal Fat	2.7	1.4	-	-
Subcutaneous Fat	2.5	1.4	-	-
Plasma	6.8	2.2	0.5	-

* Less than limit of quantitation (1.0 ppb for kidney, liver, muscle and fat; 0.5 ppb for plasma).

3. Report Number RSL 539/82914

- a) Title: An Investigation of the Nature of Radioactive Residues in the Livers of Pigs after Repeated Oral Administration of ³H-Allyl Trenbolone
Huntingdon Research Centre
- b) Report Date: October 20, 1982
- c) Name(s) and Address(es) of Investigators:
D. R. Hawkins, R. Girkin
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- d) Description of Animals: Pigs, young adult females, large white breed. 12 animals, Weights 170 kg.
- e) Route of Drug Administration: Gastric intubation.
- f) Time and Duration of Dosing: Single dose for 18 days of approximately 20 mg as a solution, 20% aqueous.
Particular isotope used – 15 mg (30 mCi) tritiated allyl trenbolone (later renamed, altrenogest). Tritium replaced hydrogen on the 6 and 7 position.
- g) Summary of Average Total Residue Concentration:
 - The nature of radioactivity in the livers of pigs sacrificed 15 and 30 days after the last of 18 daily doses of ³H-allyl trenbolone has been investigated.
 - Only 16% of the radioactivity was extractable into organic solvents, ethyl acetate (2%) and methanol (14%) after digestion of liver samples with the proteolytic enzymes, Subtilisin A. There was no appreciable increase in extractability after acid hydrolysis or β-glucuronidase treatment.
 - 84% of the radioactivity in untreated pig liver and 52% of the radioactivity in Subtilisin A digested pig liver were precipitated by trichloroacetic acid. These results indicated that a high proportion of radioactivity was irreversibly bound to macromolecules such as protein.
 - Equilibrium dialysis of 15 and 30-day liver samples using Visking tubing indicated that between 68 and 79% of the radioactivity was bound to

macromolecules of molecular weight greater than 10,000 to 20,000. Dialysis of samples using Spectrapor 3 tubing indicated that between 78 and 91% of the radioactivity was bound to molecules of molecular weight greater than about 3,500.

4. Report Number RSL **616/84177**

- a) Title: Tissue Distribution of Radioactivity and Patterns of Metabolites following Single and Multiple Doses of ³H-Altrenogest to Pigs
Huntingdon Research Centre,
- b) Report Date: June 13, 1985
- c) Name(s) and Address(es) of Investigators:
David Hawkins, I. Midgley, P.R. Dow, S. R. Biggs
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- d) Description of Animals: Pigs, young adult females, large white breed, approximately 9 months of age, approximate weight 150 kg.
- e) Route of Drug Administration: Gastric (oral) intubation. A solution in soybean oil.
- f) Time and Duration of Dosing: Dosing was carried out in three sessions; Session 1 - 1 pig, Session 2 - 14 pigs, and Session 3 - 12 pigs. Session 1 - single oral dose of 170 mg with sacrifice at 24 hours; Session 2 - 18 daily doses of 20 mg with sacrifice at 6 hours (2 pigs) (3 pigs at 5, 10, 15, and 30 days following last dose); Session 3 - daily dose of 20 mg for 3 days with slaughter 5, 10, 15, and 30 days after the last dose.
Particular isotope used - - 15 mg (30 mCi) tritiated allyl trenbolone (later renamed, altrenogest). Tritium replaced hydrogen on the 6 and 7 position.
- g) Summary of Average Total Residue Concentration:

Session 1 --

Following a single oral dose of ³H-altrenogest (170 mg=27.9 μCi/mg) to one pig, 23.7% of the radioactivity was excreted in urine during 24 hours, and the concentration of radioactivity in bile at this time represented 0.04% dose/mL. Thus, it appeared that the oral dose of ³H-altrenogest was reasonably well-absorbed and that biliary excretion played a significant role in its disposition. Thin-layer chromatographic and mass spectroscopic analytical data indicated that the principle route of biotransformation of altrenogest in this species merely involved conjugation with glucuronic acid, although several minor metabolites were also detected. At the time of sacrifice, 2.97% and 0.96% dose were present in the liver and kidneys respectively of this pig, little of which was in the form of tritiated water, and the concentration of total radioactivity in plasma was only 110 ppb, about 20% of which was in the form of tritiated water.

Session 2 --

At 6 hours after the last of 18 consecutive daily oral doses of ³H-altrenogest (nominal 20 mg/day=40.6 μCi/mg) to pigs, mean concentrations of non-volatile radioactivity in bile, liver, and kidneys represented 3815, 476, and

210 ppb, respectively. Concentrations declined with time and at 30 days after the final dose those in bile were below the limit of detection, whereas, the dose in liver and kidney represented 29 and 2 ppb, respectively at this time. Concentrations in fat and muscle were below the limit of detection (2 ppb) at all sampling times (i.e., between 5 and 30 days after the final dose).

During the first few days of the 18-day dosing period, mean concentrations of non-volatile radioactivity in plasma increased steadily and represented 16.1 ppb at 4 days. This appeared to represent the steady state level as mean concentrations changed little thereafter, and at 16 days represented 17.9 ppb. After the final dose, a peak value of 36.5 ppb occurred at 4 hours, then concentrations appeared to decline biphasically. The half-life of the terminal elimination phase was about 8 days (although this phase was not well-defined), and concentrations were below the limit of detection (i.e., 1.7 ppb) by 22 days.

Patterns of radioactive components in the plasma, uterine and bile of the pigs participating in the 18-day multiple dose study were very similar to those from the pig that received the single high dose indicating that in this dose range at least biotransformation was essentially independent of dose level.

Session 3 --

At 5 days after the last of three consecutive daily doses of ^3H -altrenogest (nominal 20 mg/day) to pigs, mean concentrations of non-volatile radioactivity in bile, liver, and kidney represented 63, 57, and 40 ppb, respectively. Concentrations declined with time and were below the limit of detection in bile at 15 days and in kidneys at 30 days; those in liver at 30 days represented 9 ppb. Again, concentrations in fat and muscle were below the limit of detection at all sampling times.

Mean concentrations of non-volatile radioactivity in plasma increased steadily during the 3-day dosing period and a peak value of 55.5 ppb occurred at 1 hour after the final dose. From this time at 4 days after the final dose - by which time they were only slightly higher than the limit of detection - concentrations appeared to decline monophasically with a half-life of 23 hours.

Thus, as might be expected, elimination of drug-related material from plasma and tissues was noticeably quicker following 3 consecutive daily oral doses of ^3H -altrenogest than it was after 18 such doses, but in both cases, concentrations of radioactivity in all tissues analyzed, with the exception of liver, were either below or just above the limit of detection at 30 days after the final dose.

5. Report Number RSL **769/89220**

- a) Title: Residue Depletion in Liver of Mature Gilts - Altrenogest
Huntingdon Research Centre,

- b) Report Date: May 3, 1989
- c) Name(s) and Address(es) of Investigators:
 Nicholas Roberts, David Cameron, David Hawkins, Ian Midgley, Alan Anderson, James Maxwell, Clive Howse
 Huntingdon Research Centre
 Huntingdon, Cambridgeshire, UK
- d) Description of Animals: Pigs, large white hybrid females, 26, approximately 7-months of age.
- e) Route of Drug Administration: Oral gavage.
- f) Time and Duration of Dosing: Given for 14 consecutive days, 15 mg (916.2 μ Ci) tritiated altrenogest in a 5 mL dose per day. Sacrificed at 6 hours, 21 days, 28 days, and 35 days following last dose.
- g) Summary of Average Total Residue Concentration: Only the liver samples were subsequently analyzed for total (3 H-altrenogest) and parent (HPLC) altrenogest. For the six animals sacrificed at 21 days of withdrawal, liver samples contained 1 ppb, 2 ppb, 3 ppb or ND (3 samples) when analyzed with the HPLC method having a stated LOD of 1 ppb.

Table 6

Group	Days Withdrawal	Concentration of Altrenogest (ppb)
1	Control	Total residues ND*
2	0 (6 hours)	479.0 \pm 6.3
3	21	31.3 \pm 4.3
4	28	24.7 \pm 6.8
5	35	18.6 \pm 4.9

* ND = Not Detected

6. Report Number: RSL 726/861541

- a) Title: Bioavailability of Altrenogest Residues in Pig Liver following Oral Administration to Rats
 Huntingdon Research Centre
- b) Report Date: February 5, 1987
- c) Name(s) and Address(es) of Investigators:
 D.R. Hawkins, I. Midgley, Philip Proctor, Alexandra Hood, Nicholas Roberts
 Huntingdon Research Centre
 Huntingdon, Cambridgeshire, UK
- d) Description of Animals: Pigs, young adult female, large white breed, body weight 138.5 kg. Rats, 12 males and 12 females, Adult Sprague Dawley CD strain, intact and bile duct-cannulated.
- e) Route of Drug Administration: Pigs - Oral Intubation, and Rats – 2-3 μ g equivalent Altrenogest/kg administered by gastric intubation.
- f) Time and Duration of Dosing: Pigs - Oral Intubation, 15 mg/day for 14 days; Rats - 10 μ g/kg altrenogest, oral single dose by gastric intubation or extracted liver homogenate from pig administered tritiated altrenogest and slaughter at 24 hours after final dose.

Particular isotope used - tritiated allyl trenbolone (later renamed, altrenogest) at a dose level similar to that used in the pig liver study. Tritium replaced hydrogen on the 6 and 7 position.

g) Summary of Average Total Residue Concentration:

During 48 hours after single oral doses of ³H-altrenogest (10 µg/kg) to bile duct-cannulated rats, most of the administered radioactivity was eliminated in the bile (mean male 63.1% dose, mean female 59.8% dose). During the same period, means of 17.1% (males) and 16.5% dose (females) were excreted in the urine, and at the time of sacrifice (48 hours post-dose) means of 2.4% (males) and 5.3% dose (females) remained in the carcass (excluding the gastro-intestinal tract). Thus, a total of about 82% of the oral dose of altrenogest was absorbed by both male and female rats.

During 48 hours after oral administration of ³H-altrenogest pig liver residues (ca. 3 µg equivalents/kg) to bile duct-cannulated rats, most of the radioactivity was excreted in the feces (mean male 71.6% dose, mean female 69.9% dose). During the same period, means of 21.0% and 8.3% dose were eliminated in bile and urine, respectively by male rats, and means of 29.1% and 8.9% dose, respectively by females. No radioactivity was detected in the remaining carcasses. These results indicate that male and female rats absorbed 29.3% and 38.0% of the administered altrenogest residues, respectively. In conclusion, between 35-47 % of the altrenogest bound residues are actually bioavailable in the rats that were dosed orally with the residues from the pig liver.

The lower absorption of the altrenogest pig liver residues was confirmed in experiments with intact rats. Thus, means of 21.1% (males) and 22.2% dose (females) were excreted in urine during 72 hours after single oral doses of ³H-altrenogest to rats, whereas in rats administered ³H-altrenogest residues, means of only 6.5% (males) and 9.2% (females) were excreted in urine during the same period. Thus, absorption of the oral dose of altrenogest amounted to about 75-80% in rats.

- h) Conclusion:
- | | |
|-----------------------------------|-----|
| Free Residues | 20% |
| Bound Residues | 80% |
| Bioavailability -- Bound Residues | 45% |

7. Report Number: RSL **819/920064**

- a) Title: An Evaluation of the Extractability of Radioactivity from the Livers of Pigs Treated with ³H-Altrenogest
Huntingdon Research Centre
- b) Report Date: January 31, 1992
- c) Name(s) and Address(es) of Investigators:
David Hawkins, L.F. Elsom
Huntingdon Research Centre
Huntingdon, Cambridgeshire, UK
- d) Description of Animals: The biological specimen used in this study was derived from the study of residue depletion in liver of mature gilts, RSL 769/89220
- e) Route of Drug Administration: Oral gavage.

- f) Time and Duration of Dosing: Given for 14 consecutive days, 15 mg of altrenogest in a 5 mL dose per day. Sacrificed at 6 hours, 21 days, 28 days, and 35 days following last dose. Isotope: tritiated altrenogest.
- g) Summary of Average Total Residue Concentration:

Table 7

Days Withdrawal	Concentration of Altrenogest (ppb)	After Correction for Bioavailability (ppb)
0 (6 hours)	597	492
21	28.3	14.2
28	31.6	14.3
35	23.6	11.8

8. Trial 98-030-P-3

- a) Title: Tissue Residue Depletion Study Following Administration of REGU-MATE to Gilts Bio Logic
- b) Report Date: July 26, 1999
- c) Name(s) and Address(es) of Investigators:
J.P. Rouillard, G. Dufour, A. de Laistre Banting
Bio Logic
Savigné sur Lathan, France
- d) Description of Animals: Twenty-two cycling gilts weighing 120 to 150 kg were used in the study.
- e) Route of Drug Administration: Oral by top dressing.
- f) Time and Duration of Dosing: 20 mg/day for 18 days.

g) Summary of Mean Altrenogest Residues in Tissues of Cycling Gilts (ppb):

Table 8

Days Withdrawal	Muscle	Liver	Kidney	Perirenal Fat	Dorsal Skin and Fat
1	4.70	85.37	9.16	59.71	55.27
7	<1.25	<1.25	<1.25	1.26	<1.25
14	<1.25	<1.25	<1.25	<1.25	<1.25
21	<1.25	<1.25	<1.25	<1.25	<1.25

CONCLUSIONS (Residue Studies)

A conservative estimate of the biologically active liver residues is as follows:

Table 9

Withdrawal	Total Ppb	Free Ppb	Bound		F + A *
			Total ppb	Available ppb	Residue ppb
6 hours	479 ± 63	96	383	172	268
21 days	31.3 ± 4.3	6.3	25.0	11.5	17.8
28 days	24.7 ± 68	4.9	19.8	8.9	13.8
35 days	18.6 ± 49	3.6	15.0	6.8	10.4

* F + A, Free residues Plus Available Bound Residue

D. Tolerance for the Marker Residue:

Based on the residue data provided in Section 4.C., tolerances for parent compound, altrenogest, of 4 ppb for liver and 1 ppb for muscle have been established.

E. Withdrawal Period:

The use of altrenogest is for the synchronization of estrus in cycling gilts. The gilts are administered 15 mg of altrenogest per day for 14 consecutive days. The assignment of a 21-day withdrawal period is consistent with the available residue data.

F. Regulatory Method for Residues:

The sponsor's validated HPLC method for altrenogest in edible tissue is on file with the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

G. User Safety Concerns:

Keep this and all medication out of the reach of children. Avoid skin contact. Wear vinyl, polyethylene, neoprene, butyl or nitrile protective gloves when handling this product. Pregnant women or women who suspect they are pregnant should not handle MATRIX

(altrenogest) Solution 0.22%. Women of childbearing age should exercise extreme caution when handling this product. Accidental absorption could lead to a disruption of the menstrual cycle or prolongation of pregnancy. Wash off accidental spillage on the skin immediately with soap and water.

The following people should not handle this product:

1. Women who are or suspect they are pregnant.
2. Anyone with thrombophlebitis or thromboembolic disorders or with a history of these events.
3. Anyone with cerebral-vascular or coronary-artery disease.
4. Women with known or suspected carcinoma of the breast.
5. People with known or suspected estrogen-dependent neoplasia.
6. Women with undiagnosed vaginal bleeding.
7. People with benign or malignant tumors which developed during the use of oral contraceptives or other estrogen-containing products.
8. Anyone with liver dysfunction or disease.

Accidental exposure:

Altrenogest is readily absorbed from contact with the skin. In addition, this oil based product can penetrate porous gloves. Altrenogest should not penetrate intact vinyl or latex gloves; however, if there is leakage (i.e., pinhole, spillage, etc.) the contaminated area covered by such occlusive materials may have increased absorption. The following measures are recommended in case of accidental exposure.

Skin Exposure: Wash immediately with soap and water.

Eye Exposure: Immediately flush with plenty of water for 15 minutes. Get medical attention.

If Swallowed: Do not induce vomiting. MATRIX (altrenogest) Solution 0.22% contains an oil. Call a physician. Vomiting should be supervised by a physician because of possible pulmonary damage via aspiration of the oil base. If possible, bring the container and labeling to the physician.

Effects of Overexposure:

There has been no human use of this specific product. The information contained in this section is extrapolated from data available on other products of the same pharmacological class that have been used in humans. Effects anticipated are due to the progestational activity of altrenogest. Acute effects after a single exposure are possible; however, continued daily exposure has the potential for more untoward effects such as disruption of the menstrual cycle, uterine or abdominal cramping, increased or decreased uterine bleeding, prolongation of pregnancy, and headaches. The oil base may also cause complications if swallowed. In addition, the list of people who should not handle this product is based upon the known effects of progestins used in humans on a chronic basis.

5. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that 15 mg altrenogest per gilt per day for 14 consecutive days when

administered in sexually mature gilts is safe and effective for synchronization of estrus in sexually mature gilts that have had at least one estrous cycle.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions for use by the lay person have been provided. Label directions provide detailed instruction in plain language. The drug product is not a controlled substance. Thus, the drug product is assigned OTC status, and the labeling is adequate for the intended use.

Under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval. The application contains investigations conducted or sponsored by the applicant that demonstrate animal safety and substantial evidence of effectiveness.

Altrenogest is under the following US patent number:

<u>U.S. Patent Number</u>	<u>Date of Expiration</u>
5,214,035	April 16, 2012

6. ATTACHMENTS:

Facsimile Labeling is attached as indicated below:

Box Label – Panel A
Box Label – Panel B
Box Label – Panel C
Box Label – Panel D
Shipper Carton Label