Approval Date: DEC 6 2001

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 140-992

REVALOR®-200 (TRENBOLONE ACETATE AND ESTRADIOL)

For increased rate of weight gain and improved feed efficiency in heifers fed in confinement for slaughter.

Sponsored by:

Intervet Inc.
P.O. Box 318
405 State Street
Millsboro, DE 19966-0318

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REVALOR®-200 (TRENBOLONE ACETATE AND ESTRADIOL) FOR HEIFERS FED IN CONFINEMENT FOR SLAUGHTER

1. GENERAL INFORMATION

NADA Number:	140-992
Sponsor:	Intervet Inc. P.O. Box 318 405 State Street Millsboro, DE 19966-0318
Generic Names:	Trenbolone Acetate and Estradiol
Trade Name:	REVALOR®-200
Marketing Status:	Over the counter (OTC)
Effect of Supplement:	Provides for the administration of REVALOR®-200 to heifers fed in confinement for slaughter.

2. INDICATIONS FOR USE

For increased rate of weight gain and improved feed efficiency in heifers fed in confinement for slaughter.

3. DOSAGE FORM(S), ROUTE(S) OF ADMINISTRATION AND RECOMMENDED DOSAGE

The dosage form is implantation. The route of administration is subcutaneous implantation on the posterior aspect of the middle one-third of the ear by means of an implant gun. The recommended dosage is one implant containing 200 mg trenbolone acetate and 20 mg estradiol. Each implant is made up of ten pellets with each pellet containing 20 mg trenbolone acetate and 2 mg estradiol. Each implant is contained in one division of a multiple dose cartridge. There are ten doses in each cartridge. The cartridge is designed to be used with a special implant gun which places the implant under the skin on the posterior aspect of the ear.

4. EFFECTIVENESS

The supplemental new animal drug application for Revalor®-200 contains data from adequate and well-controlled studies demonstrating the effectiveness of the new animal drug for the indications for use and dosage as given in Sections 2 and 3 above.

Pivotal Studies:

In dose titration studies the parameters measured are the same parameters as are measured in field investigations. The dose titration studies were conducted using a uniform protocol so that the results of the studies could be pooled and summarized. The studies were conducted in the major beef producing areas of the United States.

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The purpose of the studies was to determine the dose response for trenbolone acetate (TBA) and estradiol ($E_2\beta$) ear implants on average daily gain and feed efficiency of confined heifers. The test animals were crossbred animals of European breeds. There were 7 to 10 animals per pen depending on study location. Each treatment was replicated 8 times in all three studies. The heifers weighed between 585 (265 kg) to 639 (290 kg) lbs. when the studies were initiated. A total of 864 heifers were treated in these three dose titration studies.

Revalor® was given via ear implants. The implants were placed subcutaneously on the backside of the mid-ear. Each dose was made up of the appropriate number of pellets, with each Revalor® pellet containing 20 mg TBA and 2 mg $E_2\beta$. The control cattle were not implanted. The dosages given in each study were (TBA/ $E_2\beta$): 0/0, 200/0, 0/20, 80/8, 140/14 and 200/20. The heifers were implanted once at the initiation of the study. The length of the studies ranged between 139 and 147 days.

Average daily gain and feed efficiency data are summarized in Table 1 for each of the three dose titration studies.

TABLE 1

SUMMARY FROM THREE DOSE TITRATIONS STUDIES COMPARING THE PERFORMANCE OF CONFINED HEIFERS ON VARIOUS LEVELS OF TRENBOLONE ACETATE AND ESTRADIOL

	Location			
TBA/$E_2\beta$ (mg/mg)	**Texas**	**Colorado**	**Idaho**	**Pooled Average**
Average Daily Gain (lbs.)				
0/0	2.18	2.09	3.28	2.51
200/0	2.27	2.22	3.57	2.70
0/20	2.40	2.21	3.62	2.73
80/8	2.47	2.36	3.55	2.78
140/14	2.49	2.49	3.64	2.87
200/20	2.60	2.42	3.57	2.84
Feed Efficiency (lbs. Dry Matter/lb. Gain)				
0/0	7.20	6.89	7.01	7.03
200/0	7.02	6.41	6.57	6.66
0/20	6.89	6.43	6.73	6.69
80/8	6.78	6.27	6.77	6.60
140/14	6.69	5.98	6.57	6.42
200/20	6.47	6.03	6.63	6.38

A randomized complete block design was used for all studies and the data were pooled by analysis of variance to determine the significance of the effect of TBA/$E_2\beta$ implants on average daily gain and feed efficiency. There was a significant ($P<.05$) dose effect on both average daily gain and feed efficiency with the maximum response for both parameters plateauing at a dose of 140 mg trenbolone acetate and 14 mg estradiol. The 140 mg TBA/14 mg $E_2\beta$ and 200 mg TBA/20 mg $E_2\beta$ doses were also shown to be significantly ($P<.05$) better than 200 mg TBA alone and 20 mg $E_2\beta$ alone. Because the 200 mg TBA/20 mg $E_2\beta$ dose is not significantly better than the 140 mg TBA/14 mg $E_2\beta$ dose, the following disclaimer statement has been put on the label:

> Revalor®-200 is not better for increased average daily gain or improved feed efficiency than Revalor®-H (140 mg trenbolone acetate/14 mg estradiol) in heifers fed in confinement for slaughter.

These data are sufficient to support the claims and dosage as provided in Sections 2 and 3.

The effects of treatment on carcass parameters (yield grade, quality grade, and marbling scores) and on the incidence of liver abscesses were evaluated at slaughter. At the 200 mg TBA/20 mg $E_2\beta$ dose, a decrease in marbling scores was observed. No effect of treatment was seen in yield grade or the incidence of liver abscesses. Because of the observed decrease in marbling scores, the following statement is required on the label: studies have demonstrated that the administration of Revalor®-200 can result in decreased marbling scores when compared to non-implanted heifers.

5. TARGET ANIMAL SAFETY

The supplemental new animal drug application for Revalor®-200 references the target animal safety studies summarized in the FOI for NADA 140-992 (60 FR 4376-January 23, 1995). The data from those studies demonstrate the safety of the new animal drug for the indications for use and dosage as given in Sections 2 and 3 above.

6. HUMAN FOOD SAFETY

A. Toxicity Tests

The toxicity studies summarized in the FOI under NADA 138-612 (52 FR 24994-July 2, 1987) have met the agency's requirement for human food safety for trenbolone acetate. Allowable incremental increases of estradiol ($E_2\beta$) have been established by the agency under 21 CFR 556.240.

B. Acceptable Daily Intake

The Acceptable Daily Intake (ADI) for total residues of trenbolone acetate is 0.4 ug/kg/day.

C. Safe Concentration of Residues

Under NADA 138-612, trenbolone acetate (TBA), it was determined that the safe concentration for total residues of trenbolone was 50 ppb for muscle, 100 ppb for liver, 150 ppb for kidney and 200 ppb for fat. The total average trenbolone residues in beef liver were determined to be 43.8 ppb and 50.5 ppb at 15 and 30 days, respectively. The residues in muscle, kidney and fat were much lower.

D. Residue Depletion Studies

In addition to the three residue study summaries that follow, the supplemental new animal drug application for Revalor®-200 references the heifer tissue residue study summarized in the FOI for NADA 140-992 (60 FR 4376-January 23, 1995).

Because the original approval was based on a tissue residue depletion study conducted using 180 mg TBA/18 mg $E_2\beta$ instead of the 200 mg TBA/20 mg $E_2\beta$ approved in this supplemental application, the following tissue residue study was conducted:

Heifer and Steer Study #97U-040

A tissue residue study was conducted to determine the residues of estradiol and the two metabolites of trenbolone acetate (17α-hydroxytrenbolone (17α-TBA) and 17β-hydroxytrenbolone (17β-TBA)). This residue analysis was conducted by Dr. Donald Henricks, Clemson University, Clemson, SC (Study #97U-040).

Three (3) steers and three (3) heifers were treated with 200 mg trenbolone acetate and 20 mg estradiol. There were also one (1) control steer and one (1) control heifer in the study. All animals were sacrificed 60 days after implantation. Muscle, liver, kidney and fat samples were collected from each animal at the time of sacrifice. After collection, samples were immediately frozen in dry ice and held frozen until they were assayed for estradiol, 17α-TBA and 17β-TBA.

For estradiol levels, the purpose of the study was to determine the residue levels in the treated animals and compare them to the allowable incremental increases permitted under 21 CFR 556.240. The results are shown in Table 2.

The results of the estradiol assays from the treated and control animals were compared to the allowable incremental increases permitted under 21 CFR 556.240. When the residues of estradiol in the treated animals were compared to the naturally occurring levels in the untreated controls, they were found to be much lower than the allowable incremental increases.

TABLE 2

ESTRADIOL RESIDUES IN THE TISSUES OF REVALOR®-200 TREATED AND CONTROL STEERS AND HEIFERS

Tissue	Allowable Incremental Increase (ppt)	Estradiol (ppt) Steers		Heifers	
		60-Day Control	60-Day Treated	60-Day Control	60-Day Treated
Muscle	120	-.-*	-.-	-.-	-.-
Liver	240	-.-	41.7 ± 3.8	-.-	25.3 **
Kidney	360	-.-	26.1 ± 3.8	-.-	33.1 ± 4.6
Fat	480	-.-	86.0 ± 37.2	-.-	75.2 ± 26.5

* Less than LOQ. LOQ for estradiol is - 30 ppt muscle: 20 ppt liver and kidney: 40 ppt fat

** All but one animal had a mean concentration less than LOQ.

For trenbolone acetate residues, the purpose of the study was to determine the residue levels in the treated animals and to compare the residue levels between the two sexes (steers and heifers). The residues of 17α-TBA and 17β-TBA are reported in Table 3.

The concentration of 17α-TBA and 17β-TBA in the four tissues on Day 60 after implanting are similar to the concentrations on Days 15 and 30 after implanting as reported in the following study (Study #4667-01-07-95) in steers only. There are no sex-related (steers vs. heifers) differences in the concentrations of 17α-TBA and 17β-TBA in the four tissues.

The 60-day tissue residue values are not statistically different for steers and heifers.

TABLE 3

RESIDUES OF 17α-TBA AND 17β IN THE TISSUES OF REVALOR®-200 TREATED STEERS AND HEIFERS

Trenbolone 17α (ppt)				
	Steers		Heifers	
Tissue	60-Day Control	60-Day Treated	60-Day Control	60-Day Treated
Muscle	-.-*†	95.5 **	-.-	-.-
Liver	-.-	1430.0 ± 486.0	-.-	1587.0 ± 1041.0
Kidney	-.-	128.8 ±12.5	-.-	324.1 ± 203.7
Fat	-.-	-.-	-.-	-.-
Trenbolone 17β (ppt)				
	Steers		Heifers	
Tissue	60-Day Control	60-Day Treated	60-Day Control	60-Day Treated
Muscle	-.-	97.9 ± 24.4	-.-	97.1 ± 17.7
Liver	-.-	480.6 ± 179.0	-.-	514.8 ± 46.2
Kidney	-.-	152.2 ± 31.8	-.-	-.-
Fat	-.-	344.0 ± 152.0	-.-	338.1 ± 50.1

* -.- Values are less than the LOQs for the respective tissues.

** All but one animal had a mean concentration less than LOQ.

† LOQs for the Revalor®-200 study: muscle: 50 ppt TBA-α and TBA-β; fat: 100 ppt TBA-α and TBA-β; liver: 200 ppt TBA-α and TBA-β; kidney: 100 ppt TBA-α and TBA-β.

Steer Study #4667-01-07-95

A tissue residue study was conducted to determine the residues of estradiol and the two metabolites of trenbolone acetate (17α-hydroxytrenbolone (17α-TBA) and 17β-hydroxytrenbolone (17β-TBA)). This residue analysis was conducted by Dr. Donald Henricks, Clemson University, Clemson, SC (Study #4667-01-07-95).

Eight (8) steers were treated with 200 mg trenbolone acetate and 20 mg estradiol. There were also four (4) control steers in the study. In the treatment group, four steers were sacrificed 15 days after treatment and the remaining four were sacrificed 30 days after implantation. In the control group, two steers were sacrificed 15 days after treatment and the remaining two were sacrificed 30 days after the treatment group was implanted. Muscle, liver, kidney and fat samples were collected from each animal at the time of sacrifice. After collection, samples were immediately frozen in dry ice and held frozen until they were assayed for estradiol, 17α-TBA and 17β-TBA.

For estradiol levels, the purpose of the study was to determine the residue levels in the treated animals and compare them to the allowable incremental increases permitted under 21 CFR 556.240. The results are shown in Table 4.

TABLE 4

ESTRADIOL RESIDUES IN THE TISSUES OF REVALOR®-200 TREATED AND CONTROL STEERS

Tissue	Allowable Incremental Increase (ppt)	Estradiol (ppt)			
		15-Day Control	15-Day Treated	30-Day Control	30-Day Treated
Muscle	120	-.-*	13.4 ± 2.4	-.-	13.6 ± 3.7
Liver	240	-.-	84.8 ± 23.9	-.-	28.6
Kidney	360	61.2 ± 9.1	60.4 ± 20.7	98.6 ± 15.7	64.9 ± 22.2
Fat	480	-.-	67.1 ± 16.9	-.-	59.4 ±20.5

* Less than LOQ. LOQ for estradiol is - 5 ppt muscle and fat: 24 ppt liver and kidney

The results of the estradiol assays from the treated and control animals were compared to the allowable incremental increases permitted under 21 CFR 556.240. When the residues of estradiol in the treated animals were compared to the naturally occurring levels in the untreated controls they were found to be much lower than the allowable incremental increases.

For trenbolone acetate residues, the purpose of the study was to determine the residue levels in the treated animals and compare them to the residues incurred with the approved Finaplix® implant (200 mg trenbolone acetate alone). The residues of 17α-TBA and 17β-TBA are reported in Table 5.

TABLE 5

RESIDUES OF 17α-TBA AND 17β-TBA IN THE TISSUES OF REVALOR®-200 TREATED STEERS AND STEERS TREATED WITH 200 MG OF TRENBOLONE ACETATE ALONE (FINAPLIX®).

Trenbolone 17α (ppt)								
	Revalor®-200				Finaplix®			
Tissue	15-Day Control	15-Day Treated	30-Day Control	30-day Treated	15-day Control	15-day Treated	30-day Control	30-day Treated
Muscle	-.-*†	19.14± 3.25	-.-	-.-	-.-**	-.-	-.-	-.-
Liver	-.-	1550± 932	-.-	802.2± 239.1	-.-	4023.1± 2415.3	-.-	1774.8± 470.3
Kidney	-.-	178.0± 45.2	-.-	167.2± 23.7	-.-	-.-	-.-	-.-
Fat	-.-	60.2± 11.7	-.-	43.9± 11.5	-.-	-.-	-.-	-.-
Trenbolone 17β (ppt)								
	Revalor®- 200				Finaplix®			
Tissue	**15-Day Control**	**15-Day Treated**	**30-Day Control**	**30-Day Treated**	**15-Day Control**	**15-Day Treated**	**30-Day control**	**30-Day Treated**
Muscle	-.-	279.4± 38.5	-.-	233.8± 47.2	-.-	211.3± 39.5	-.-	138.6± 63.1
Liver	-.-	239.7± 83.0	-.-	216.1± 30.1	-.-	761.7± 161.2	-.-	497.7± 67.8
Kidney	-.-	175.7± 21.5	-.-	129.9± 4.9	-.-	387.4± 35.3	-.-	337.1± 66.0
Fat	-.-	378.3± 61.6	-.-	260.2± 81.1	-.-	846.6± 73.2	-.-	660.9± 127.4

* -.- Values are less than the LOQs for the respective tissues.

† LOQs for the Revalor®-200 study: muscle: 30 ppt TBA-α and TBA-β; fat: 30 ppt TBA-α and TBA-β; liver: 125 ppt TBA-α and TBA β; kidney: 125 ppt TBA-α and TBA-β.

** LOQs for the Finaplix® study: muscle: 15 ppt TBA-α, 30 ppt TBA-β; fat: 30 ppt TBA-α and TBA-β; liver: 125 ppt TBA-α and TBA-β; kidney: 250 ppt TBA-α and TBA-β.

The residues of Revalor®-200 treated animals are less at 30 days post-implantation than at 15 days post-implantation. The liver, kidney, and fat trenbolone β residues resulting from the use of Revalor®-200 were less than the liver, kidney, and fat residues of trenbolone β resulting from the use of Finaplix® (Table 5). For muscle, the residues of trenbolone β are higher in Revalor®-200 treated steers than in Finaplix® treated steers and the difference is statistically significant ($P<.05$). However, residue values determined using the validated RIA can be directly related to total residue equivalents and when this is done, the muscle residues resulting from both the use of Revalor®-200 and Finaplix® are less than half of the consumption - adjusted ADI for muscle, 80 ppb. With the exception of liver, the residues of trenbolone α are higher in Revalor®-200 treated steers than in Finaplix® treated steers. However, since most of the trenbolone α residues resulting from the use of Finaplix® are less than the LOQ for the method, the statistical significance of any observed differences cannot be determined.

Additionally, to evaluate potential gender differences, residue data previously collected to support the approval of Revalor®-S were reevaluated.

A tissue residue study was conducted to determine the residues of estradiol and the two metabolites of trenbolone acetate (17 α-hydroxytrenbolone and 17β-hydroxytrenbolone). This study was conducted by Dr. Don Henricks at Clemson University, Clemson, S. C.. Eight (8) steers were treated with 140 mg trenbolone acetate and 28 mg estradiol. An additional eight (8) steers were treated with 200 mg trenbolone acetate. There were also four (4) control steers in the study. In each of the treatment groups, four steers were sacrificed 15 days after treatment and the other four steers were sacrificed 30 days after the initial implantation. Muscle, fat, liver and kidney samples were collected from each animal on each of the sacrifice dates. After collection, the samples were immediately frozen in dry ice and held frozen until they were assayed for 17α-hydroxytrenbolone, 17β-hydroxytrenbolone and estradiol residues. The following two tables summarize the results from this study. As residue levels were similar at the 15 and 30 day sampling, the results in the following two tables are averaged across both sampling dates for the treated animals. Estradiol residues were assayed in only the control steers and steers treated with 140 mg TBA and 28 mg estradiol.

In Table 6, the results from the estradiol tissue assays are summarized. The results of the estradiol assays from the treated and control animals are compared with the acceptable safe incremental increases above naturally occurring levels established in 21 CFR Section 556.240. The estradiol levels from the treated and control animals were many times lower than the acceptable safe incremental levels. Since the acceptable safe incremental increases of estradiol exceed the estradiol levels found in the treated steers by such a wide margin, it was concluded that no pre-slaughter withdrawal period and no withholding restrictions were necessary. Thus there is no need for a regulatory tissue assay method for estradiol.

TABLE 6

ESTRADIOL LEVELS OF TREATED AND CONTROL STEERS COMPARED TO ESTABLISHED SAFE INCREMENTAL LEVELS

Estradiol (ppt)			
Tissue	Acceptable Increments	Revalor®-S (No Withdrawal)	Untreated Controls
Muscle	120	-.-*	-.-
Fat	480	16.1 ± 2.6	6.1 ± 1.7
Kidney	360	-.-	-.-
Liver	240	-.-	-.-

* Less than LOQ. Limit of quantitation (LOQ) for muscle 6 ppt; fat 6 ppt; kidney 25 ppt; and liver 25 ppt.

The residues of 17α-hydroxytrenbolone and 17β-hydroxytrenbolone are reported in Table 7. When the residues of the two trenbolone metabolites are compared between the two implant groups, the residues from the implant containing 140 mg TBA/28 mg $E_2\beta$ are consistently lower than the residues from the steers implanted with only trenbolone acetate (200 mg). Thus, implanting cattle with the combination product (REVALOR®-S) gave trenbolone residues that were lower compared to the residues when trenbolone is implanted alone. Additional information on the residues of trenbolone acetate can be found in the Freedom of Information Summary for NADA 138-612 (52 FR 24994-July 2, 1987).

TABLE 7

17αHYDROXYTRENBOLONE (TB-α) AND17β-HYDROXYTRENBOLONE (TB-β) RESIDUES IN STEER TISSUES

Tissue				
Treatment (Implant)	Muscle	Fat	Liver	Kidney
TB-α (ppt)				
Control	-.-*	-.-	-.-	-.-
140 mg TBA/28 mg E_2β	-.-	-.-	285.3 ± 14.8	-.-
200 mg TBA	-.-	126.9 ± 102.3	2899.0 ± 2009.7	-.-
TB-β (ppt)				
Control	-.-**	-.-	-.-	-.-
140 mg TBA/28 mg E_2β	75.6 ±14.6	176.6 ± 48.1	199.9 ± 50.1	-.-
200 mg TBA	175.0 ± 62.3	753.8 ± 138.2	629.7 ± 181.7	362.4 ± 56.0

* Less than LOQ. TB-α Limit of quantitation (LOQ) for muscle 15 ppt; fat 30 ppt; liver 125 ppt; and kidney 250 ppt.

** Less than LOQ. TB-β Limit of quantitation (LOQ) for muscle 30 ppt; fat 30 ppt; liver 125 ppt; and kidney 250 ppt.

E. Withdrawal Period

Residues of estradiol in heifers and steers treated with Revalor®-200 are several times less than the allowable incremental increases permitted under 21 CFR 556.240. When RIA-determined residues are equated to total residue equivalents, the residues of trenbolone in each of the edible tissues are less than the allowable safe concentrations for these tissues. Additionally, there are no gender-related differences in incurred residues in steers and heifers treated with either 140 mg TBA + 28 mg E_2® (15 and 30 day data) or 200 mg TBA + 20 mg E_2® (60-day data). Therefore, the use of Revalor®-200 qualifies for a zero withdrawal in both heifers and steers.

F. Regulatory Method

Revalor®-200 qualifies for a zero withdrawal and, as such, a regulatory analytical method for residues is not required.

7. 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 demonstrates that an implant containing 200 mg trenbolone acetate and 20 mg estradiol when used in heifers fed in confinement for slaughter is safe and effective for the claims of increased rate of weight gain and improved feed efficiency.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions for use by the layman have been provided and the product will retain its over-the-counter marketing status.

Under section 512(c)(2)(F)(iii) of the FFDCA, this approval for food producing animals qualifies for THREE years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the supplemental application and conducted or sponsored by the applicant. The three years of marketing exclusivity applies only to the use of the product (REVALOR®-200); containing 200 mg trenbolone acetate and 20 mg estradiol for which the supplemental application was approved.

8. LABELING

Five (5) pages of labeling are attached as follows:

1. Package Insert (front)
2. Package Insert (back)
3. Box of 10 x 10 cartridge implants
4. Box of 10 x 100 dose packages
5. 4 x 1000 dose packages