

TRENBOLONE ACETATE

IDENTITY

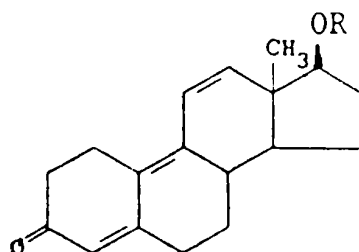
Chemical name: Trenbolone
17 β -hydroxyestra-4,9,11-trien-3-one
4,9,11-estratrien-17 β -ol-3-one
17 β -hydroxy-19-norandrosta-4,9,11-trien-3-one
19-norandrosta-4,9,11-trien-17 β -ol-3-one

Trenbolone acetate

17 β -acetoxy-3-oxoestra-4,9,11-triene
17 β -acetoxyestra-4,9,11-triene-3-one
3-oxo-17 β -hydroxy-4,9,11-estratrieneacetate

Synonyms: Trenbolone
trienbolone
trienolone

Structural formula:



R = H (trenbolone)
R = COCH₃ (trenbolone acetate)

Molecular formula: C₁₈H₂₂O₂ (trenbolone), C₂₀H₂₄O₃ (trenbolone acetate)
Molecular weight: 270.38 (trenbolone), 312.39 (trenbolone acetate)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

	<u>Trenbolone</u>	<u>Trenbolone acetate</u>
Appearance:	Pale yellow, crystals	crystals
Melting point:	183 - 186°C	96 - 97°C
Optical rotation:	[α] _D ²⁰ = +19° (c=0.45 in ethanol) +36.8° (c=0.37 in methanol)	
UV_{max}:	239, 340.5nm	

(Windholz, 1983)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Trenbolone acetate (TBA) is a synthetic steroid with anabolic properties. It is administered as a subcutaneous implant to the base of the ear and is used to improve body weight, feed conversion and nitrogen retention in cattle 60-90 days or more before the intended date of slaughter. It is used alone or in combination with another hormonally active ingredient. The ear, along with any residual drug, is discarded at slaughter.

Dosages:

Finaplix (300 mg TBA) = heifers
 Torelor (200 mg TBA + 40 mg estradiol-17 β) = steers
 Revalor (140 mg TBA + 20 mg estradiol-17 β) = calves

RADIOLABELED RESIDUE STUDIES

General

Trenbolone acetate upon entering the circulatory system is rapidly hydrolysed to its free active form, trenbolone (TBOH). In the rat the 17 β -epimer is the major metabolite. In the bovine species the 17 α -epimer is the major metabolite occurring in the excreta, bile and liver; the 17 β -epimer is the major metabolite occurring in muscle. (Jouquey, et al., 1983)

Calves

Twelve calves, 6 castrates and 6 females, weighing 150-200 kg each received an implant containing 200 mg of [6,7-³H]-trenbolone acetate (TBA). Six animals were sacrificed at each of 15 and 30 days after implantation. Samples of liver, kidney, muscle, fat, and bile were taken for analysis at the time of slaughter. Measurements of radioactivity were made on both intact and freeze-dried samples. Tissues were oxidized prior to measurement of radioactivity.

The tissue concentrations of radioactivity (total and non-volatile) are given in Tables I and II, respectively.

Table I. Concentration of Total Radioactivity in Tissues (μ g/kg)

<u>Tissue</u>	<u>Time After Implantation</u>	
	<u>15 day</u>	<u>30 day</u>
Liver	43.8 \pm 21.7	50.5 \pm 11.4
Kidney	16.4 \pm 5.6	21.8 \pm 5.1
Muscle	2.41 \pm 0.65	3.23 \pm 0.50
Fat	2.45 \pm 1.15	2.40 \pm 0.88
Bile	1163 \pm 1046	741 \pm 148

Table II. Concentration of Non-Volatile Radioactivity
in Tissues (µg/kg)

<u>Tissue</u>	<u>Time After Implantation</u>	
	<u>15 day</u>	<u>30 day</u>
Liver	42.5 + 22.0	49.3 + 10.9
Kidney	15.1 + 6.3	20.5 + 5.2
Muscle	1.58 + 0.49	2.64 + 0.36
Fat	2.38 + 1.36	2.31 + 0.74
Bile	1073 + 918	736 + 151

Samples of liver were homogenized and portions of the homogenates were extracted with diethyl ether or ethyl acetate. Some portions were incubated with β -glucuronidase overnight prior to extraction. The amount of extractable radioactivity in liver is given in Table III.

Table III. Radioactivity Extracted from Liver
(% of Total Sample Radioactivity)

	<u>Untreated Homogenate</u>		<u>Glucuronidase-Treated</u>	
	<u>Ether</u>	<u>EtOAC</u>	<u>Ether</u>	<u>EtOAC</u>
15 day	11.1 + 3.1	14.9 + 3.3	25.9 + 5.5	28.9 + 5.1
30 day	8.1 + 2.1	11.7 + 2.5	18.3 + 3.2	21.4 + 3.9

(Hawkins, et al., 1984)

Heifers

Two heifers were implanted with 300 mg ^3H -TBA and slaughtered after 60 days. Total residues averaged 32.2 and 2.4 µg/kg in the liver and muscle, respectively. The percent of radioactivity recovered from the tissues by ether after enzymatic hydrolysis was only 15 and 5 in the liver and muscle, respectively. Based on in vitro studies with liver homogenate and a relatively low CBI (covalently binding index 3.79 - 7.36), the authors postulate that the non-extractable radioactivity may represent residues bound to other structures than DNA. (Hoffman et al., 1984)

RESIDUE STUDIES

Steers

Five groups of steers, 6 per group, were used in this study. Group I served as untreated controls. The 24 steers in groups II-V were implanted with 200 mg of trenbolone acetate (TBA) + 40 mg of estradiol-17 β (E2 β). One group was slaughtered at each of the following times after implantation: 15 days, 30 days, 60 days, and 75 days. Control steers were slaughtered at each of these time points.

Free and conjugated 17 β -trenbolone (17 β -TBOH), free and conjugated 17 α -trenbolone (17 α -TBOH), and free and conjugated estradiol-17 β were determined in muscle, liver, kidney, fat, and plasma. Free estrone was measured in liver and fat and conjugated 17 α -TBOH and conjugated E2 α were determined in urine. Analyses were done by an HPLC-RIA method.

Tissue residues of free and conjugated 17 β - and 17 α -TBOH are given in Tables IV-VII: Levels reported without standard deviations are at or below the detection limit of the method.

Table IV. Free 17 β -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
Control	17	33		8	21
15-Day	254 + 62	467 + 162		78 + 41	392 + 147
30-Day	272 + 80	323 + 131		67 +	293 + 171
60-Day	108 + 29	180 + 105		78 + 24	120 + 106
75-Day	71 + 32	83 + 52		52 +	111 + 86

Table V. Conjugated 17 β -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
Control	34	56		15	34
15-Day	66	1110 + 568		35	27
30-Day	43	772 + 618		36	31
60-Day	38	695 + 337		33	32
75-Day	43	401 + 177		33	20

Table VI. Free 17 α -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
Control	36	41		50	38
15-Day	0	213 + 71		95 + 44	74 + 20
30-Day	9	226 + 80		76 + 8	62 + 19
60-Day	41	89 + 26		24 +	60 +
75-Day	40	39 +		23	55

Table VII. Conjugated 17 α -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
Control	13	47		39	41
15-Day	21	1918 + 864		386 + 282	59
30-Day	10	1708 + 758		210 + 44	36
60-Day	27	908 + 664		143 + 27	52
75-Day	16	656 + 331		182 + 51	16

The concentration of free 17 β -TBOH in muscle, liver, and fat are comparable to each other, while in the kidney the concentration is down near the detection limit. Detectable levels of conjugated 17 β -TBOH were found only in liver.

Free 17α -TBOH could only be detected in liver until 60 days after implantation and in kidney and fat until 30 days after implantation. Detectable amounts of conjugated 17α -TBOH were found in liver and kidney. [Arts, et al., 1986 (a)]

Heifers

Twenty-four heifers weighing approximately 280 kg were implanted with Finaplix, which contains 300 mg of TBA. Six heifers were slaughtered at each of the following times after implantation: 15, 30, 60, and 75 days. Free and conjugated 17β -TBOH, 17α -TBOH, and E 2β were determined in muscle, liver, kidney, fat, and plasma. Conjugated 17α -TBOH and conjugated E 2α were determined in urine.

Tissue residues of free and conjugated 17β - and 17α -TBOH are given in Tables VIII-XI:

Table VIII. Free 17β -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
15-Day	526 + 237	528 + 162		530 + 310	1091 + 546
30-Day	645 + 328	440 + 148		445 + 195	1021 + 535
60-Day	152 + 24	253 + 67		340 + 72	345 + 164
75-Day	187 + 103	110 + 63		145 + 66	158 + 109

Table IX. Conjugated 17β -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
15-Day	60	1031 + 650		179 + 62	31
30-Day	75	972 + 470		167 + 38	46
60-Day	34	909 + 268		144 + 34	31
75-Day	97 + 34	499 + 176		33	30

Table X. Free 17α -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
15-Day	73 + 78	440 + 192		144 + 87	152 + 48
30-Day	102 + 106	286 + 78		155 + 47	113 + 54
60-Day	60	63 + 30		57	93 + 19
75-Day	42	71 + 25		26	70 + 27

Table XI. Conjugated 17 α -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	<u>Muscle</u>	<u>Liver</u>	<u>Tissue</u>	<u>Kidney</u>	<u>Fat</u>
15-Day	75	4255 + 1729		464 + 353	62
30-Day	59	2920 + 1130		309 + 176	60
60-Day	20	1699 + 755		200 + 103	40
75-Day	81	1572 + 733		242 + 107	44

Standard deviations given are the absolute standard deviations. Values with no standard deviations are values that are at or below the detection limit of the assay.

The concentration of free 17 β -TBOH in muscle, liver, and kidney are comparable at 15 days after implantation. The concentration in fat was almost double the other tissues. At 60 days after implantation, the concentration of free 17 β -TBOH had significantly decreased as compared to levels at 15 or 30 days after implantation.

Detectable levels of conjugated 17 β -TBOH were only found in liver and kidney. Free 17 α -TBOH was found in muscle and kidney until 30 days after implantation, and in liver and fat throughout the testing period. (Arts, et al., 1986(b))

Calves

Twenty-four calves, twelve male and twelve female, were implanted with Revalor (140 mg trenbolone acetate + 20 mg estradiol); three calves of each sex were slaughtered at the following withholding periods: 15, 30, 50 and 70 days. Eight calves were used as controls. Two males and two females were slaughtered at withholding periods of 30 and 70 days. Liver and kidney were analyzed by RIA for free and conjugated 17 α -TBOH and 17 β -TBOH. Muscle was analyzed for total (free plus conjugate) 17 α -TBOH and 17 β -TBOH. The results are summarized in Tables XII and XIII. (Roberts and Cameron, 1986)

Table XII. 17 β -TBOH Mean Tissue Concentrations In Calves Implanted with REVALOR (ng/kg)

	<u>Muscle</u>	<u>Liver</u>		<u>Kidney</u>	
	<u>Total*</u>	<u>free</u>	<u>conjugate</u>	<u>free</u>	<u>conjugate</u>
Control	29.5 + 10.9	90.5 + 51.0	50.8 + 24.0	34.8 + 9.84	33.3 + 8.6
15-Days	237 + 87.5	414 + 178	404 + 198	23 + 208	240 + 43.7
30-Day	228 + 108	908 + 404	366 + 112	586 + 52.7	207 + 47.6
50-Day	261 + 91.6	787 + 413	366 + 95.7	226 + 156	198 + 50.4
70-Day	219 + 125	763 + 226	436 + 56.9	389 + 211	252 + 61.5

*Sum of free and conjugate 17 α -TBOH.

Table XIII. 17 α -TBOH Mean Tissue Concentrations In Calves Implanted with REVALOR (ng/kg)

	<u>Muscle</u> <u>Total*</u>	<u>free</u>	<u>Liver</u> <u>conjugate</u>	<u>free</u>	<u>Kidney</u> <u>conjugate</u>
Control	30.3 + 17.0	62.4 + 25.6	46.8	19.6 + 15.9	34.8 + 11.6
15-Days	81.2 + 39.6	982 + 245	1202 + 598	322 + 184	312 + 283
30-Day	105 + 43.7	1078 + 353	754 + 315	196 + 90.8	221 + 34.0
50-Day	66.6 + 32.5	683 + 301	584 + 226	193 + 54.6	139 + 37.7
70-Day	44.2 + 16.5	540 + 149	733 + 206	142 + 37.7	91.6 + 1.92

* Sum of free and conjugate 17 α -TBOH.

METHODS OF RESIDUE ANALYSIS

General

Several analytical techniques have been utilized to quantitate levels of trenbolone in plasma, excreta and tissues. Although economical, TLC procedures are limited to sensitivities of 10-100 $\mu\text{g}/\text{kg}$. HPLC and GC-MS techniques extend the quantitation levels to 1-10 $\mu\text{g}/\text{kg}$. RIA techniques further extend the quantitation levels to 0.1-1 $\mu\text{g}/\text{kg}$ and are able to measure both the 17 α -TBOH and 17 β -TBOH metabolites. (Hoffman and Ryan, 1978) (Hoffman and Oettel, 1976) (Jouquey, et al., 1983)(O'Keefe, 1984a) (O'Keefe, 1984b)

RIA

Homogenates of the tissues are extracted with toluene:ether (7:3) in order to separate the free and conjugated steroids. The conjugated steroids are incubated with glucuronidase and sulphatase and the free steroids are extracted with toluene:ether. The steroids are purified by solid phase chromatography, separated by HPLC and quantitated by RIA. The reported detection levels in tissues for free 17 β -TBOH, conjugated 17 β -TBOH, free 17 α -TBOH and conjugated 17 α -TBOH are 70, 75, 60, and 75 ng/kg, respectively. (Arts, et al., 1986(b))

APPRAISAL

The maximum total drug-related residues resulting from implants containing 200 mg of trenbolone acetate are approximately 50 $\mu\text{g}/\text{kg}$ and 3 $\mu\text{g}/\text{kg}$ in liver and muscle, respectively. Approximately 25 per cent of the total residue is extractable from glucuronidase-treated liver by ether or ethyl acetate. From the studies of Hawkins, et al., (1984) and Hoffman, et al., (1984), the percent of total residue extracted from muscle at withholding periods shorter than 60 days can be estimated to be 10. Using total residue concentrations of 3 and 50 $\mu\text{g}/\text{kg}$ in muscle and liver, the concentrations of soluble residues of trenbolone acetate are calculated to be 0.3 and 12.5 $\mu\text{g}/\text{kg}$ in muscle and liver, respectively. The majority of residue from trenbolone acetate is not extractable by organic solvents and is somehow "bound" in the tissues. Any safety evaluation of trenbolone acetate must consider the significance of these "bound" residues.

The residue levels in muscle and liver of 17 β -TBOH, 17 α -TBOH and their conjugates at 30 days after implantation are summarized in Table XIV for steers, heifers and calves. Although the dosage levels and the withholding periods are not the same in the three experiments, the residue data are useful in determining the qualitative nature of the soluble residues in the tissues.

For muscle tissue, the steer data indicates that almost all of the soluble residue is 17 β -TBOH. In heifers the 17 α -TBOH is above the detection level; the free 17 α -TBOH is approximately one third of the 17 β -TBOH. For liver, the free 17 β -TBOH exceeds the free 17 α -TBOH in liver in steers and heifers; however, when the conjugates are included, the combined 17 β -TBOH is 30-70% of the combined 17 α -TBOH.

In summary, the primary residue in muscle is 17 β -TBOH but the free 17 α -TBOH is not an insignificant percent of the residue. Together all free residues total .3-.7 μ g/kg in muscle.

Table XIV. Muscle and Liver Concentrations in Steers, Heifers and Calves Implanted with Trenbolone Acetate (ng/kg)

	<u>Muscle</u>		<u>Liver</u>	
	<u>17β-TBOH</u>	<u>17α-TBOH</u>	<u>17β-TBOH</u>	<u>17α-TBOH</u>
Steer: free	272	-	323	226
conjugate	-	-	772	1708
Heifer: free	645	102	440	286
conjugate	-	-	972	2920
Calves: free	228*	105*	908	1078
conjugate			366	754

* These values represent the sum of free and conjugate 17 β -TBOH and 17 α -TBOH, respectively.

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