

**TRENBOLONE ACETATE**

**IDENTITY**

Chemical name:

Trenbolone

17 $\beta$ -hydroxyestra-4,9,11-trien-3-one  
4,9,11-estratrien-17 $\beta$ -ol-3-one  
17 $\beta$ -hydroxy-19-norandrosta-4,9,11-trien-3-one  
19-norandrosta-4,9,11-trien-17 $\beta$ -ol-3-one

Trenbolone acetate

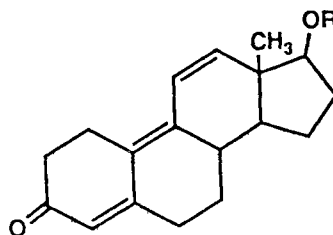
17 $\beta$ -acetoxy-3-oxoestra-4,9,11-triene  
17 $\beta$ -acetoxyestra-4,9,11-triene-3-one  
3-oxo-17 $\beta$ -hydroxy-4,9,11-estratrieneacetate

Synonyms:

Trenbolone

trienbolone  
trienolone

Structural formula:



R = H (trenbolone)  
R = COCH<sub>3</sub> (trenbolone acetate)

Molecular formula:

C<sub>18</sub>H<sub>22</sub>O<sub>2</sub> (trenbolone), C<sub>20</sub>H<sub>24</sub>O<sub>3</sub> (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:	$[\alpha]_D^{20} = +19^\circ$ (c=0.45 in ethanol)	+36.8° (c=0.37 in methanol)
UV <sub>max</sub> :	239, 340.5 nm	
(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.

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

This monograph will explore the residues of trenbolone acetate when used in combination with estradiol and other estradiol-containing products to determine if an increase in either 17 $\beta$ -trenbolone (17 $\beta$ -TBOH) or 17 $\alpha$ -trenbolone (17 $\alpha$ -TBOH) levels is produced. This determination will be based on three studies in steers, 2 studies in heifers and 1 study in calves.

#### Dosages

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

### 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 Torelor which is 200 mg of trenbolone acetate (TBA) + 40 mg of Estradiol-17 $\beta$  (E2 $\beta$ ). 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 $\beta$ -TBOH, free and conjugated 17 $\alpha$ -TBOH, and free and conjugated estradiol-17 $\beta$  were determined in muscle, liver, kidney, fat, and plasma. Free estrone was measured in liver and fat and conjugated 17 $\alpha$ -TBOH and conjugated E2 $\alpha$  were determined in urine. Analyses were done by an HPLC-RIA method.

Tissue residues of free and conjugated 17 $\beta$ - and 17 $\alpha$ -TBOH are given in Tables I-IV: Levels reported without ranges are at or below the detection limit of the method.

The detection limit was considered to be 70 ng/kg and was defined as the residue level which could be reliably determined (although considerably lower levels are detectable using the analysis method).

**TABLE I. Free 17 $\beta$ -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>
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 II. Conjugated 17 $\beta$ -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>
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 III. Free 17 $\alpha$ -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>
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 IV. Conjugated 17 $\alpha$ -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>
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 $\beta$ -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 $\beta$ -TBOH were found only in liver.

Free 17 $\alpha$ -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 17 $\alpha$ -TBOH were found in liver and kidney [(Arts, et al., 1986 (a))].

In a second study, twenty-four steers, weighing between 400-450 kg, were divided into four groups with six animals per group. The animals of Group I were implanted once with Torelor (40 mg estradiol + 200 mg trenbolone acetate) and slaughtered at 60 days after implantation. The animals of Group II, III, and IV were implanted twice (60 day interval between implantation) with Torelor and slaughtered 15, 30, and 60 days, respectively, after the second implantation. It should be noted in this study that when Torelor is reimplanted, it is reimplanted in the opposite ear.

The contents of free and conjugated  $17\beta$ -TBOH and free and conjugated  $17\alpha$ -TBOH were determined in muscle, liver, kidney, fat, and plasma. The determination of the different steroids in the biological matrices was performed by using an HPLC-RIA procedure. (Heister, M., 1986). This was the same analytical method used in the previous study with the same limit of detection.

Tissue residues of free and conjugated  $17\beta$ - and  $17\alpha$ -TBOH are given in Tables V-VIII:

**TABLE V. Free  $17\beta$ -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)**

Time of slaughtering after first implantation after second implantation	60 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	188 $\pm$ 55	295 $\pm$ 88	351 $\pm$ 103	282 $\pm$ 85
Liver	103 $\pm$ 37	219 $\pm$ 111	99 $\pm$ 47	48
Kidney	256 $\pm$ 76	402 $\pm$ 96	188 $\pm$ 50	163 $\pm$ 45
Fat	631 $\pm$ 395	1149 $\pm$ 473	636 $\pm$ 131	826 $\pm$ 269

**TABLE VI. Conjugated  $17\beta$ -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)**

Time of slaughtering after first implantation after second implantation	60 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	35	35	37	18
Liver	551 $\pm$ 182	976 $\pm$ 330	779 $\pm$ 330	330 $\pm$ 130
Kidney	82 $\pm$ 37	105 $\pm$ 22	84 $\pm$ 17	63 $\pm$ 23
Fat	15	21	12	16

**TABLE VII. Free  $17\alpha$ -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)**

Time of slaughter after first implantation after second implantation	60 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	70 $\pm$ 46	61 $\pm$ 56	36	48
Liver	141 $\pm$ 60	211 $\pm$ 108	115 $\pm$ 42	47
Kidney	35	43	65 $\pm$ 19	48
Fat	20	24	77 $\pm$ 16	62 $\pm$ 20

**TABLE VIII. Conjugated  $17\alpha$ -TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)**

Time of slaughtering after first implantation after second implantation	60 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	63	80 $\pm$ 37	88 $\pm$ 21	87 $\pm$ 21
Liver	1731 $\pm$ 475	3085 $\pm$ 2183	4652 $\pm$ 1513	2055 $\pm$ 575
Kidney	183 $\pm$ 104	191 $\pm$ 90	163 $\pm$ 81	95 $\pm$ 18
Fat	29	35	76 $\pm$ 35	60

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 contents of free  $17\beta$ -TBOH in the muscle, liver, kidney and fat were significantly higher after the bi-sequential implantation when compared to the concentrations after the mono-implantation. Detectable levels of conjugated  $17\beta$ -TBOH are only found in the liver and kidney.

The contents of free  $17\alpha$ -TBOH are mainly found in the liver. The majority of  $17\alpha$ -TBOH residues are found in the conjugated portion with significant levels primarily in the liver and kidney. (Arts, et al., 1986(b)).

In a third study, eight steers, weighing approximately 280 kg, were simultaneously implanted with Finaplix-S (TBA - 140 mg) and Synovex-S (progesterone - 200 mg + E2 $\beta$  - 20 mg) in the left and right ears, respectively. Four steers were slaughtered 15 days after implantation and the other four steers were slaughtered 30 days after implantation. Samples of fat and muscle were collected for analysis of E2 $\beta$ , progesterone, and  $17\alpha$ -TBOH and  $17\beta$ -TBOH, and samples of kidney and liver were collected for analysis of  $17\alpha$ -TBOH and  $17\beta$ -TBOH only.

An RIA procedure was utilized to determine the concentration of  $17\alpha$ -TBOH and  $17\beta$ -TBOH in muscle, kidney, liver and fat. It should be noted that the unbound residues of trenbolone- $17\alpha$  and trenbolone- $17\beta$  were measured in the muscle and fat, and the free plus the glucuronide and sulfate conjugates were measured in the liver and kidney.

Tissue residues of  $17\beta$ -TBOH and  $17\alpha$ -TBOH are given in Tables IX-X:

**TABLE IX.  $17\beta$ -TBOH Mean Tissue Concentrations in Steers Simultaneously Implanted with FINAPLIX and SYNOVEX-S (ng/kg)**

	<u>Muscle</u>	<u>Liver</u> *	<u>Tissue</u>	<u>Kidney</u> *	<u>Fat</u>
15-Day	147 $\pm$ 15	491 $\pm$ 39		< 250	421 $\pm$ 53
30-Day	241 $\pm$ 40	596 $\pm$ 108		< 250	505 $\pm$ 52

\* - The values for the liver and kidney represent the free plus conjugated portion, and the values for the muscle and fat represent only the free portion.

**TABLE X.  $17\alpha$ -TBOH Mean Tissue Concentrations in Steers Simultaneously Implanted with FINAPLIX and SYNOVEX-S (ng/kg)**

	<u>Muscle</u>	<u>Liver</u> *	<u>Tissue</u>	<u>Kidney</u> *	<u>Fat</u>
15-Day	< 15	1128 $\pm$ 242		< 250	51 $\pm$ 14
30-Day	< 15	1045 $\pm$ 165		< 250	< 30

\* - The values for the liver and kidney represent the free plus conjugated portion, and the values for the muscle and fat represent only the free portion.

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 contents of the free plus conjugated portion of  $17\beta$ -TBOH in the liver and the free portion in the fat and muscle were significantly higher thirty days after implantation as compared to 15 days after implantation. No detectable residues of  $17\beta$ -TBOH were found in the kidney.

The only significant residues of the free plus conjugated portion of  $17\alpha$ -TBOH were found in the liver. (Herschler, R.C. 1988).

### 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  $17\beta$ -TBOH,  $17\alpha$ -TBOH, and e2 $\beta$  were determined in muscle, liver, kidney, fat, and plasma. Conjugated  $17\alpha$ -TBOH and conjugated e2 $\alpha$  were determined in urine.

Tissue residues of free and conjugated 17 $\beta$ - and 17 $\alpha$ -TBOH are given in Tables XI-XIV:

**TABLE XI. Free 17 $\beta$ -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 XII. Conjugated 17 $\beta$ -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 XIII. Free 17 $\alpha$ -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 XIV. Conjugated 17 $\alpha$ -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 $\beta$ -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 $\beta$ -TBOH had significantly decreased as compared to levels at 15 or 30 days after implantation.

Detectable levels of conjugated 17 $\beta$ -TBOH were only found in liver and kidney. Free 17 $\alpha$ -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(c)).

In a second study, thirty heifers weighing approximately 270 kg each were divided into five groups with six animals per group. The heifers in Group I served as a control group

and the animals in Group II-V were implanted twice with Finaplix (300 mg TBA) (60 day interval between implantation). The animals were slaughtered 0, 15, 30, and 60 days, respectively, after the second implantation. It should be noted in this study that when Finaplix is re-administered it is re-implanted in the opposite ear.

The contents of free and conjugated  $17\beta$ -TBOH, free and conjugated  $17\alpha$ -TBOH and free and conjugated estradiol- $17\beta$  were determined in muscle, liver, kidney, fat, and plasma. The determination of the different steroids in the biological matrices was performed by using an HPLC-RIA procedure. (Heister, M., 1986).

Tissue residues of free and conjugated  $17\beta$ - and  $17\alpha$ -TBOH are given in Tables XV-XVIII:

**TABLE XV. Free  $17\beta$ -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)**

Time of slaughtering after first implantation	60 days	75 days	90 days	120 days
after second implantation	0 days	15 days	30 days	60 days
Muscle	164 $\pm$ 143	460 $\pm$ 196	210 $\pm$ 70	268 $\pm$ 116
Liver	95 $\pm$ 71	331 $\pm$ 150	212 $\pm$ 84	181 $\pm$ 125
Kidney	176 $\pm$ 162	586 $\pm$ 221	259 $\pm$ 129	156 $\pm$ 91
Fat	523 $\pm$ 502	2258 $\pm$ 980	716 $\pm$ 188	511 $\pm$ 224

**TABLE XVI. Conjugated  $17\beta$ -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)**

Time of slughtering after first implantation	60 days	75 days	90 days	120 days
after second implantation	0 days	15 days	30 days	60 days
Muscle	48	25	26	23
Liver	385 $\pm$ 378	1172 $\pm$ 571	1091 $\pm$ 353	1029 $\pm$ 480
Kidney	69	137 $\pm$ 76	123 $\pm$ 23	128 $\pm$ 23
Fat	14	8	10	17

**TABLE XVII. Free  $17\alpha$ -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)**

Time of slaughtering after first implantation	60 days	75 days	90 days	120 days
after second implantation	0 days	15 days	30 days	60 days
Muscle	53	96 $\pm$ 24	44	45
Liver	97 $\pm$ 54	247 $\pm$ 134	256 $\pm$ 78	187 $\pm$ 115
Kidney	37	110 $\pm$ 51	72 $\pm$ 30	44
Fat	21	60	86 $\pm$ 32	77 $\pm$ 19

**TABLE XVIII. Conjugated  $17\alpha$ -TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)**

Time of slaughtering after first implantation	60 days	75 days	90 days	120 days
after second implantation	0 days	15 days	30 days	60 days
Muscle	64	59	78 $\pm$ 11	74
Liver	1052 $\pm$ 1026	4178 $\pm$ 1791	3232 $\pm$ 462	2376 $\pm$ 968
Kidney	116 $\pm$ 78	245 $\pm$ 88	339 $\pm$ 199	212 $\pm$ 71
Fat	14	25	57	57

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 highest concentration of free 17 $\beta$ -TBOH was found in the fat and was more than three times the residue level found in muscle, liver and kidney, which all contained comparable levels. Detectable levels of conjugated 17 $\beta$ -TBOH were found in the liver.

The only significant concentrations of free 17 $\alpha$ -TBOH and conjugated 17 $\alpha$ -TBOH were found in the liver and kidney, with levels of up to 4,000 ng/kg in the liver.

In almost all cases, the concentration of either free or conjugated 17 $\alpha$ -TBOH or 17 $\beta$ -TBOH was the highest in the heifers slaughtered 15 days after the re-implantation.

### 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 $\alpha$ -TBOH and 17 $\beta$ -TBOH. Muscle was analyzed for total (free plus conjugate) 17 $\alpha$ -TBOH and 17 $\beta$ -TBOH. There was no significant sex-related difference in TBOH levels. The combined results are summarized in Tables XIX and XX. (Roberts and Cameron, 1986)

**TABLE XIX - 17 $\beta$ -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>
15-Day	237 + 87.5	414 + 178	404 + 198	423 + 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 $\beta$ -TBOH.

**TABLE XX - 17 $\alpha$ -TBOH Mean Tissue Concentrations In 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>
15-Day	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 $\alpha$ -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 ppb. HPLC and GC-MS techniques extend the quantitation levels to 1-10 ppb. RIA techniques further extend the quantitation levels to 0.1-1 ppb and are able to measure both the 17 $\alpha$ -TBOH and 17 $\beta$ -TBOH metabolites. All analyses presented in the previous residue studies were conducted using RIA procedures. (Heister, 1986; Hoffman and Ryan, 1978; Hoffman and Oettel, 1976; Jouquey, et al., 1983; O'Keefe, 1984a and 1984b)



### Radioimmunoassay 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 sulfatase 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 $\beta$ -TBOH, conjugated 17 $\beta$ -TBOH, free 17 $\alpha$ -TBOH and conjugated 17 $\alpha$ -TBOH are 70, 75, 60, and 75 ppt, respectively. (Arts, et al., 1986(c)).

### APPRAISAL

The residue levels in muscle and liver of 17 $\beta$ -TBOH, 17 $\alpha$ -TBOH and their conjugates at 30 days after implantation or reimplantation are summarized in Table XXI for steers, heifers and calves. Although the dosage levels and the withholding periods are not the same in the six experiments, the residue data are useful in determining the qualitative nature of the soluble residues in the tissues.

For muscle tissue, the data collected in steers, heifers and calves indicate that almost all of the soluble residue is 17 $\beta$ -TBOH. In the muscle, the ratio of 17 $\beta$ -TBOH to 17 $\alpha$ -TBOH ranges from about 10 for the steer to 3 for the heifer to 2 for the calf. For liver tissue, the amount of free 17 $\beta$ -TBOH and free 17 $\alpha$ -TBOH in the steers, heifers and calves are comparable; however, when the conjugates are included, the combined 17 $\beta$ -TBOH is 30-60% of the combined 17 $\alpha$ -TBOH.

In summary, the primary residue in muscle is 17 $\beta$ -TBOH but the free 17 $\alpha$ -TBOH is not an insignificant percent of the residue. In the liver, the primary extractable residue is 17 $\alpha$ -TBOH, but the 17 $\beta$ -TBOH makes up a significant portion of the combined extractable residues. Together all free residues total 0.25-0.75 ppb in the muscle and 0.2-2.0 ppb in the liver.

TABLE XXI - Muscle and Liver Concentrations in Steers, Heifers and Calves Thirty Days after Implanted with Trenbolone Acetate (ng/kg)

	<u>Muscle</u>		<u>Liver</u>	
	<u>17<math>\beta</math>-TBOH</u>	<u>17<math>\alpha</math>-TBOH</u>	<u>17<math>\beta</math>-TBOH</u>	<u>17<math>\alpha</math>-TBOH</u>
Steer : free <sup>1</sup>	272	9	323	226
conjugate <sup>1</sup>	43	10	772	1708
free <sup>2</sup>	351	36	99	115
conjugate <sup>2</sup>	37	88	779	465
free <sup>3</sup>	241	<15	596*	1045*
conjugate <sup>3</sup>	—	—	—	—
Heifer: free <sup>4</sup>	645	102	440	286
conjugate <sup>4</sup>	75	59	972	2920
free <sup>5</sup>	210	44	212	256
conjugate <sup>5</sup>	26	78	1091	3232
Calves: free <sup>6</sup>	228*	105*	908	1078
conjugate <sup>6</sup>	—	—	366	754

- 1 - Steers were implanted with Torelor (200 mg TBA + 40 mg E2 $\beta$ ).
- 2 - Steers were implanted twice with Torelor and the values reflect 30 days after reimplantation.
- 3 - Steers were simultaneously implanted with Finaplix-S (140 mg TBA) and Synovex-S (200 mg progesterone + 20 mg E2 $\beta$ ).
- 4 - Heifers were implanted with Finaplix (300 mg TBA).
- 5 - Heifers were implanted twice with Finaplix (300 mg TBA) and the values reflect 30 days after reimplantation.
- 6 - Calves were implanted with Revalor (140 mg TBA + 20 mg E2 $\beta$ ).

\* These values represent the sum of free and conjugate 17 $\beta$ -TBOH and 17 $\alpha$ -TBOH, respectively.

#### REFERENCES

- Arts, C.J.M., van Baak, M.J., Huisman, I.J., and van Weerden Ir.E.J. (1986a).  
Residue studies in steers implanted with TORELOR. Unpublished report ILOB-report 561a. Submitted to FAO by Roussel-UCLAF, Romainville, France.
- Arts, C.J.M., van Baak, M.J., de Jong, J., and van Weerden Ir.E.J. (1986b).  
Residue studies in steers after a bi-sequential implantation with TORELOR.  
Unpublished report ILOB-report 572. Submitted to FAO by Roussel-UCLAF,  
Romainville, France.
- Arts, C.J.M., van Baak, M.J., Huisman, I.J., and van Weerden Ir.E.J. (1986c).  
Residue studies in heifers implanted with FINAPLIX. Unpublished report ILOB-report 560a. Submitted to FAO by Roussel-UCLAF, Romainville, France.
- Arts, C.J.M., van Baak, M.J., de Jong, J., and van Weerden Ir.E.J. (1986d).  
Residue studies in heifers after bi-sequential implantation with FINAPLIX.  
Unpublished report ILOB-report 571. Submitted to FAO by Roussel-UCLAF,  
Romainville, France.
- Heister, M. (1986). HPLC-RIA and HPLC-TLC determination of free and conjugated  
17 $\beta$ -trenbolone, 17 $\alpha$ -trenbolone and estradiol-17 $\beta$  in bile of steers and heifers.  
Unpublished report, ILOB, Wageningen, Netherlands. Submitted to FAO by Roussel-  
UCLAF, Romainville, France.
- Herschler, R.C., Kushinsky, S., Kearley, E.O., and Henricks, D. (1988).  
Concentrations of estradiol-17 $\beta$ , progesterone and trenbolone in tissues of feedlot  
steers following simultaneous implantation of Finaplix and Synovex-S. Study No.  
IAS 1139-C878, Syntex Research, Palo Alto, California, and Roussel-UCLAF, Paris,  
France. Submitted to FAO by Roussel-UCLAF, Paris, France.
- Jouquey, A., Mouren, M., and Salmon, J. (1983). Analytical methods for  
trenbolone. In: Anabolics in Animal Production, Office International des  
Epizooties, Paris, pp. 423-441.
- O'Keefe, M. (1984a). Tissue levels of the anabolic agents, trenbolone and  
zeranol, determined by radioimmunoassays. Proc. of the Symp. on the Analysis of  
Steroids, Szeged, Hungary. Akademiai Kiado, Budapest (tubls.). Ed. S. Jorog  
(1985).
- O'Keefe, M. (1984b). Trenbolone levels in tissues of trenbolone acetate-implanted  
Steers: radioimmunoassay determination using different antisera. Br. Vet. J. 140,  
592-599.
- Roberts, N.L. and Cameron, D.M. (1986). Steroid Levels in tissues of veal calves  
following implantation with Implix BM/BF and/or Revalor lactose. Unpublished  
report No. RSL/686 for Huntington Research Centre, Huntington, Cambridgeshire,  
England. Submitted to FAO by Roussel-UCLAF, Romainville, France.
- Windholz, M. ed. (1983). The Merck Index 10th Edition. Rahway, N.J., Merck and Co.