ESTRADIOL-17β, PROGESTERONE AND TESTOSTERONE

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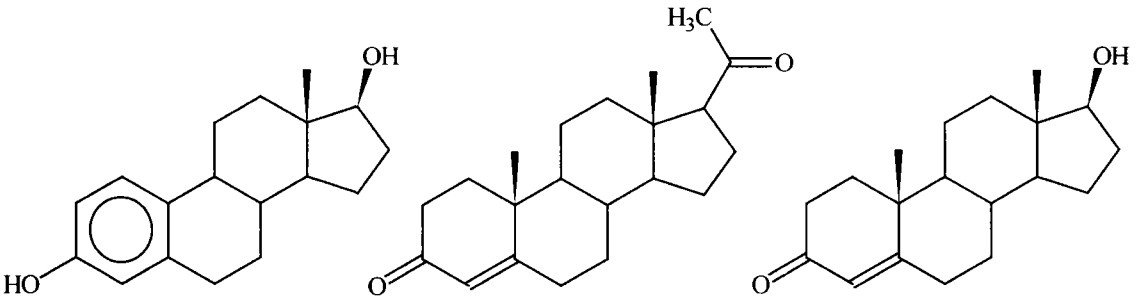
IDENTITY

	Estradiol-17β	**Progesterone**	**Testosterone**
Chemical name:	estra-1,3,5(10)-triene-3,17β-diol	pregn-4-ene-3,20-dione Δ4-pregnene-3,20-dione	17β-hydroxyandrost-4-en-3-one Δ4-androsten-17β-ol-3-one
Synonyms:	estradiol-17β	Corpus luteum hormone Luteohormone	trans-testosterone

Structural formula:



Molecular formula:	$C_{18}H_{24}O_2$	$C_{21}H_{30}O_2$	$C_{19}H_{28}O_2$
Molecular weight:	272.37	314.45	288.41
Appearance:	White crystals or crystalline powder	Crystals	needles
Melting point:	173-179° C	α-form: 127-131°C β-form: 121°C	155°C
Optical rotation:	$[\alpha]_D^{25}$ = +76 to +83° in dioxane	$[\alpha]_D^{25}$ = +172 to +182° (c=2 in dioxane)	$[\alpha]_D^{25}$ = +109° (c=4 in alcohol)
UV_{max}:	225, 280 nm	240 nm	238 nm
Purity (USP grade):	97-103%	>98%	>97%

DOSAGE

Estradiol-17β, alone or combinations with progesterone, testosterone or trenbolone acetate are given to animals to improve their rate of weight gain and feed efficiency. Administration is by subcutaneous implantation in the ear. The ear, along with any residual drug is discarded at slaughter. If estradiol-17β benzoate or testosterone propionate is used

instead of the un-esterified forms, the esters are rapidly hydrolysed in the animal after release from the implant. This report uses data obtained using the implants characterised in Table 1.

Table 1 Composition of certain implants used for growth promotion and target animals for their use

Product Name	Composition of implants [mg/implant]						Target Animals
	Estradiol	estradiol-benzoate	testosterone	testosterone propionate	progesterone	trenbolone-acetate	
Compudose	24 45						cattle
Synovex S		20			200		steers
Synovex H		20		200			heifers
Synovex C		10			100		calves
Steer-oid		20			200		steers
Heifer-oid		20		200			heifers
Implix BM	20				200		steers
Implix BF	20		200				heifers
Torelor	40					200	steers
Revalor lactose	20					140	calves
Revalor G	8					40	steers
Revalor S	24					120	steers
Revalor H	14					140	heifers
Finaplix-S						140	steers

The use of Torelor as well as the concomitant or sequential use of Implix/Revalor is currently not authorised

INTRODUCTION

The use of endogenous anabolic agents in farm animals, which has been reviewed (e.g., Weiert Velle, 1976), is summarised below.

Estradiol-17β

Estradiol-17β is produced by the ovaries and the foetal placenta. In cows, production rates are very high in late pregnancy (several hundred mg every 24 hours). Plasma levels, which are present in the ng/L level in non-pregnant cows, are much higher (2-6 μg/L) in late pregnancy. Ruminants metabolise estradiol-17β to the 17α-isomer, which possesses very low estrogenic activity and is the major urinary metabolite. Biliary excretion is quantitatively the most important route for estradiol-17β.

Relative estrogenic potencies for estradiol-17β and its main metabolites in cattle have been estimated using the immature mouse uterus test and are shown in Table 2.

Table 2. Relative estrogenic potencies for estradiol-17β and the main metabolites of in cattle

Estrogen	Subcutaneous route	Oral route
Estrone	1	1
Estradiol-17β	3.41	2.01
Estradiol-17α	0.05	0.07

Progesterone

Progesterone is the main sex hormone produced by the corpus luteum and in cattle it is also responsible for the maintenance of pregnancy. Furthermore, it is an important precursor for the biosynthesis of androgens and corticosteroids and is also present in males and castrates. Conversion to androgens and excretion via faeces is an important route in ruminants.

Testosterone

The major source of testosterone is the testis. Small amounts are also secreted by the adrenals and the ovaries. Production rates in bulls are approximately 40-50 mg every 24 hours. Male cattle show a considerable diurnal variation in secretion. In the bovine male, testosterone is largely converted to the less active epitestosterone, which is mainly excreted in the faeces.

RESIDUES IN ANIMALS AND THEIR EVALUATION

Studies of the SYNOVEX® implants

Studies with implants containing ^{14}C labelled hormones

Several early reports, starting about 1973, deal specifically with a description of the total radioactivity appearing in plasma, urine and faeces collected daily over a period of 14 days post implantation with ^{14}C-labeled Synovex S and Synovex H preparations. Radioactivity was also determined in biopsy samples of selected muscle and fat tissues obtained three days post implantation, and in muscle, fat, liver and kidney obtained at slaughter 14 days post implantation (FDA/CVM NADA file 9-576, Vol. 3, p. 199; Vol. 4, p. 211; Vol. 4, p. 262; Vol. 4, p. 318). Four animals were implanted with different combinations of labelled and unlabelled steroid as shown in Table 3.

Table 3. Composition of SYNOVEX® implants used in radioisotope studies in cattle

Animal	Amount of steroid implanted								
	^{14}C-Estradiol benzoate		Unlabelled estradiol benzoate	^{14}C-testosterone propionate		Unlabelled testosterone propionate	^{14}C-progesterone		Unlabelled progesterone
	mCi	mg	mg	mCi	mg	mg	mCi	mg	mg
Heifer A			20	4.98	200				
Heifer B	3.09	20							200
Steer C			20				5.36	200	
Steer D	2.95	20							200

Table 4 summarises the recovery of radioactivity from urinary and faecal excreta form implanted animals, together with the radioactivity recovered from the implant. In general, the amount of radioactivity appearing in plasma was very low. The levels seen in the heifers were, on the average, lower than those seen in the steers, which could be explained by a lower release rate from the implants.

Analyses of biopsy samples removed three days after implantation showed that there was a lag period in the accumulation of hormones released from the implant site. Only radioactive progesterone could be detected in fat at this time. After 14 days, progesterone, of the three steroids, produced the majority of radioactive residues in all tissues. The smallest proportion was distributed to muscle, as shown in Table 5. The major route of excretion was via the faeces.

Table 4. **Recovery of radioactivity in urinary and faecal excreta and from the implants of implanted animals.**

Animal	Labeled Molecule	Recovery of implanted dose (%)		
		Urinary excretion	Faecal excretion	Recovered from implant
Heifer A	Testosterone	0.78	6.4	89
Heifer B	Estradiol-17β	3.4	5.4	79
Steer C	Progesterone	0.96	27	64
Steer D	Estradiol-17β	5.5	9.3	54

Table 5 **Distribution of radioactivity in tissues of heifers and steers 14 days post implantation with radiolabelled hormones in the proportions of Synovex S and Synovex H**

Tissue		ng equivalents / kg of tissue			
		Heifer		Steer	
		A	B	C	D
		Testosterone propionate	Estradiol-17β	Progesterone	Estradiol-17β
Muscle-	Neck	400	40	2500	30
	Rump	400	35	1900	40
Liver		7000	660	54000	660
Kidney		1700	680	16000	830
Fat-	Neck	2400	380	45000	400
	Rump	2600	380	45000	240

A significant fraction of the radioactivity was water-soluble and the metabolites responsible were probably conjugates of the respective hormones. The glucuronides appeared to be the predominant conjugates and were found in muscle, liver and kidney. Sulfate conjugates were also found in muscle, liver and kidney.

The radioactivity appearing in liver, kidney and muscle of Heifer A, which had received the implant with labelled testosterone propionate, was evenly distributed between the free and conjugated forms. In fat, the radioactivity appeared predominantly in the free form. In Heifer B, which received labelled estradiol in the implant, the unconjugated free estradiol fraction was predominant in fat and liver. The unconjugated fraction was only slightly higher than the conjugated fraction in muscle; in kidney the conjugated fraction was slightly higher than the unconjugated fraction. In Steer D, which had also received labelled estradiol in the implant, the unconjugated fractions dominated in fat and muscle. However, the conjugated fractions were slightly higher in liver and kidney.

The free steroid fraction predominated in the fat and muscle of Steer C, which had received labelled progesterone. In liver and kidney, the two fractions were more equally distributed with the unconjugated fraction still being slightly higher than the conjugated fraction. Unchanged progesterone was the main labelled compound in fat and was also the major component of the unconjugated fractions in muscle, liver and kidney. Between 10-20% of the total conjugated radioactivity was not hydrolysed by β-glucuronidase, sulfatase or solvolysis. The results are summarised in Table 6.

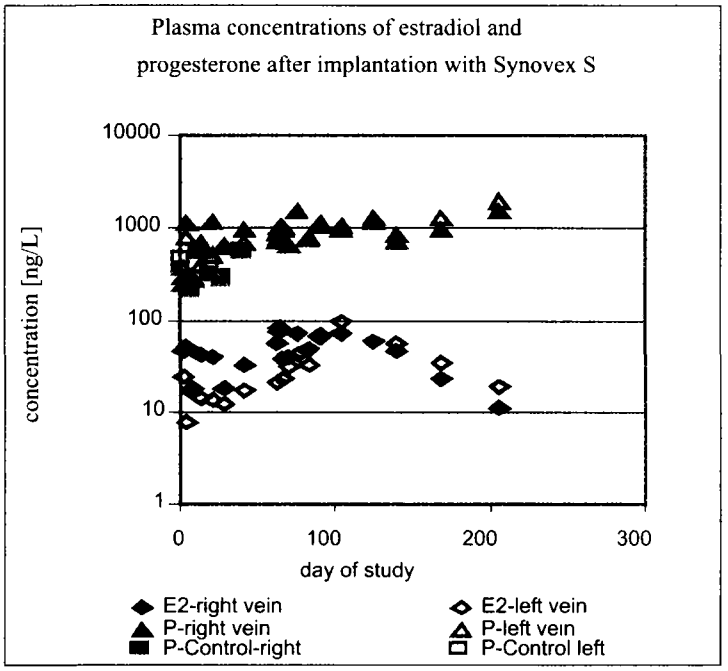
Table 6. Distribution of radioactivity into water-soluble fraction ("conjugates") of treated cattle

Tissue		Water soluble residues of ^{14}C-testosterone propionate (%)	Water soluble residues of ^{14}C estradiol benzoate (%)		Water soluble residues of ^{14}C-progesterone (%)
		Heifer A	Heifer B	Steer D	Steer C
Muscle	Neck	50	40	35	21
	Rump	39	43	33	31
Liver		56	27	57	48
Kidney		56	63	66	41
Fat	Neck	13	10	18	4
	Rump	4	3	4	8

Effects of implantation / re-implantation of Synovex S on plasma levels in steers

Nine steers were treated with one Synovex implant in the right ear on study day 1 and re-implanted in the left ear on study day 63. Samples of blood were collected from the right and left jugular veins on various days after treatment and the concentrations of estradiol-17β and progesterone in plasma were determined (Lee and Massey, 1989). The outcomes of this study are illustrated in Figure 1. The number of sampling points was too limited to fully describe the kinetic and dynamic effects on plasma hormone levels resulting from the implantation. However, it seems that estrogen levels showed a distinct maximum at about 40 days after the second implantation (99.2 ng/L of estradiol in plasma from the left jugular vein on day 104 of the study). The highest level observed after the first implantation was observed on day 63 of the study, immediately before the re-implantation and was 57.8 ng/L. For progesterone the corresponding maxima were obtained on day 21 (1190 ng/L) and on day 206 (1910 ng/L).

Figure 1. Plasma concentrations of estradiol and progesterone after implantation with SYNOVEX S



Methods used for steroid quantification including methods validation

A radio-immunoassay method for the determination of estrone, estradiol-17β and progesterone was developed and progressively validated over several years (Kushinsky and Mirrasoul, 1979). The range of concentrations over which the assay was validated was consistent with the endogenous concentrations of hormones. The current review relies primarily on the progress report of the method by Kushinsky and Mirrasoul and on subsequent addenda to that report. The very sophisticated procedure, which has been described in full detail, starts with the extraction of the residues from tissues followed by removal of fat, separation of neutral and phenolic compounds, sulfatation, partitioning to separate progesterone from neutral hydroxy-steroids, and TLC to separate estrone from estradiol. When testosterone was later included in the procedure, hormone extracts were also purified by TLC prior to RIA analysis. Recovery was determined using ^{3}H-labelled hormones (procedural recovery was 40-60%). A typical example of the results of the estimation of the procedural recovery is given in Table 7. From recovery experiments with fortified control samples the slopes of regression lines linking the concentration found to the concentration added can be calculated. It can be seen from the results given in Table 8 that the method slightly overestimates the concentrations of the free hormones with the exception of progesterone in muscle, liver and kidney, which are slightly underestimated.

Table 7. Recovery (%) for the RIA analysis of estrone, estradiol-17β and progesterone

Tissue	Recovery (%) and Standard deviation (n=12)					
	Estrone		Estradiol-17β		Progesterone	
	Mean	SD	Mean	SD.	Mean	SD.
Muscle	58.1	2.01	63.9	5.27	61.5	2.31
Fat	51.1	3.70	57.5	3.09	42.3	2.58

Table 8. Performance factors for the RIA analysis of estrone, estradiol-17β and progesterone

Tissue	Working range and slope of regression line								
	Estrone			Estradiol-17β			Progesterone		
	Range		Slope	Range		Slope	Range		Slope
Muscle	2	27	1.11	0.94	13.5	1.14	650	2650	0.84
Fat	12	62	1.02	1.2	26	1.08	2800	18800	1.03
Kidney	10	60	1.17	17	117	1.19	770	4770	0.91
Liver	3	28	1.07	3	28	1.25	540	4540	0.95

Highly specific antisera were used for the radioimmunoassay. The RIA was typically conducted with 2 tubes for the unknown, 3 tubes per standard and 3 tubes for procedural controls. Examination of the details of the method description suggests that the procedure is unlikely to be capable of including the conjugated forms of the hormones in the determination of residues. Results were corrected for recovery. Procedural blanks were very low (1.2-1.4 picograms(pg)/test tube for estradiol, 1.9-2.5 pg/test tube for estrone, and 2.7-3.1 pg/test tube for progesterone). Using pooled tissue extracts, the RIA was further validated in a later study by demonstrating that nearly identical results were obtained using a GC-MS technique.

All analyses of incurred tissues were run as duplicates and, sometimes, as several replicates. The intra-assay variability with fortified tissues is concentration-dependent as seen in Figure 2.

In the process of further validation, tissues of six hormonally untreated finished steers were analysed in duplicate under intra-assay and inter-assay conditions, respectively. The results are compared in Figure 3.

Figure 2. **Variability intra-assay of sixfold estimates of steroid hormones in steer tissues**

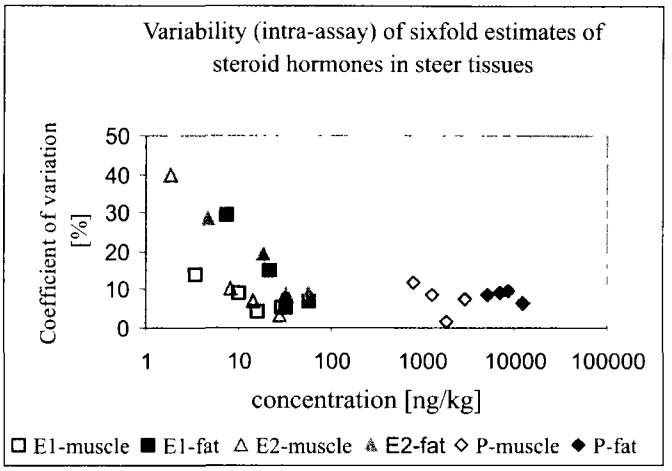
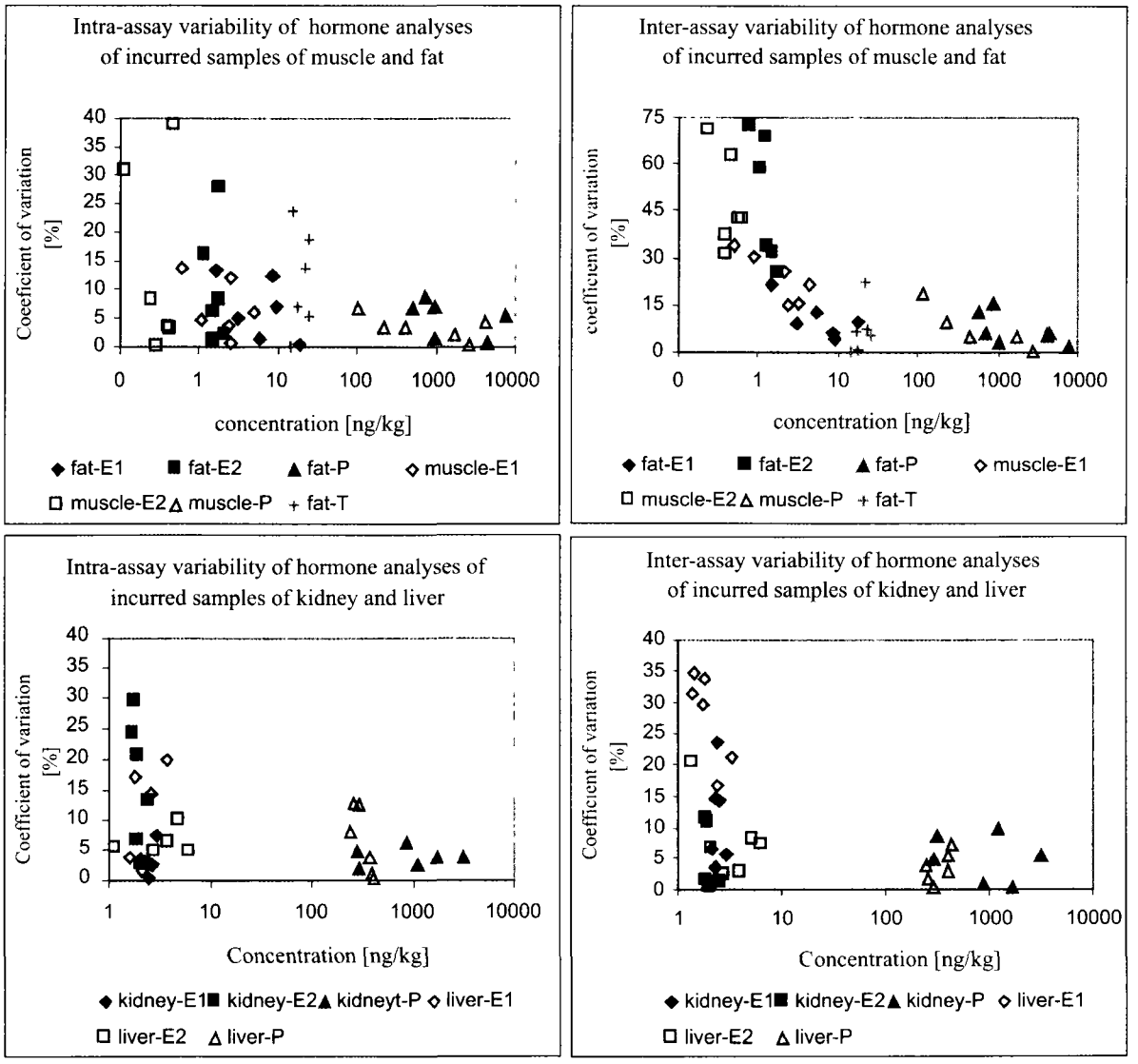


Figure 3. **Intra-assay variability of hormone analyses of incurred samples of muscle, fat, liver and kidney**



In all residue studies where this method was used all incurred tissues were analysed at least in duplicate. This database is excellently suited to investigate the precision of the method under routine conditions. The following figures 4a - 4c illustrate this performance characteristic as calculated by the current reviewer on the example of steer tissues and the three hormones progesterone, estradiol-17β and estrone.

Figure 4. Precision of duplicate estimates of progesterone, estradiol-17β and estrone in steer tissues

Figure 4a

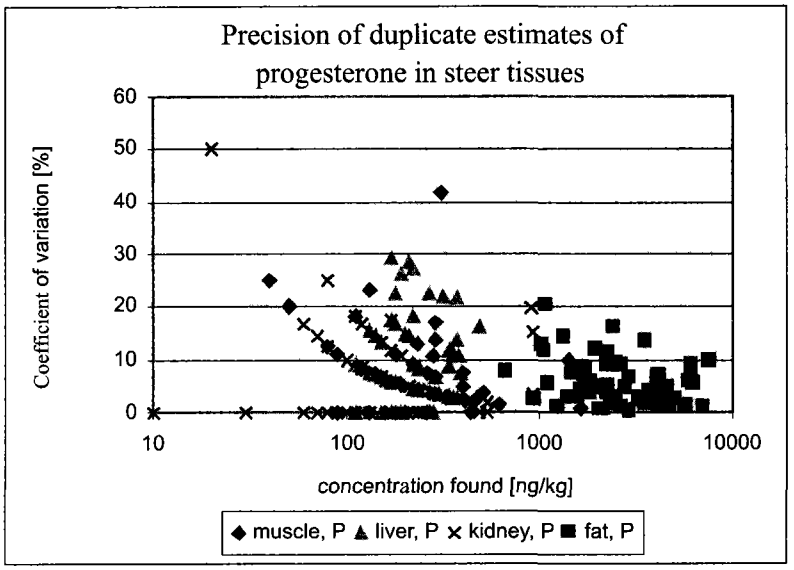


Figure 4b

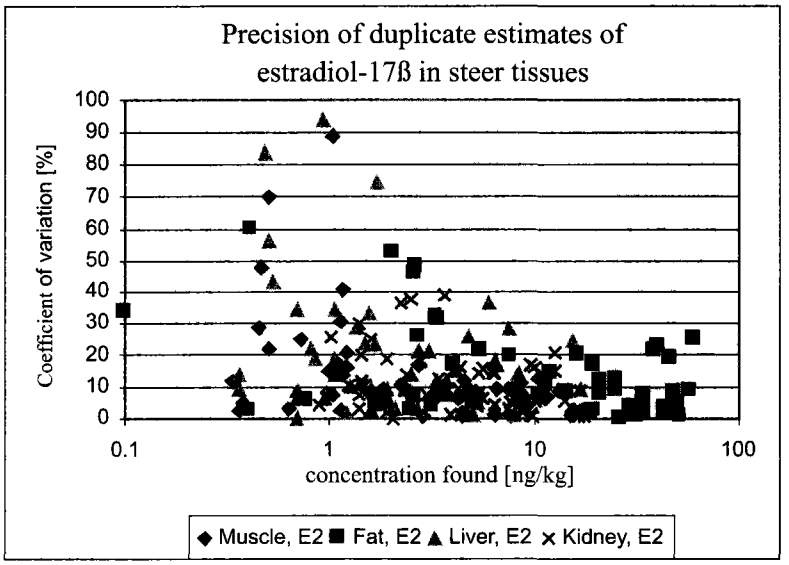
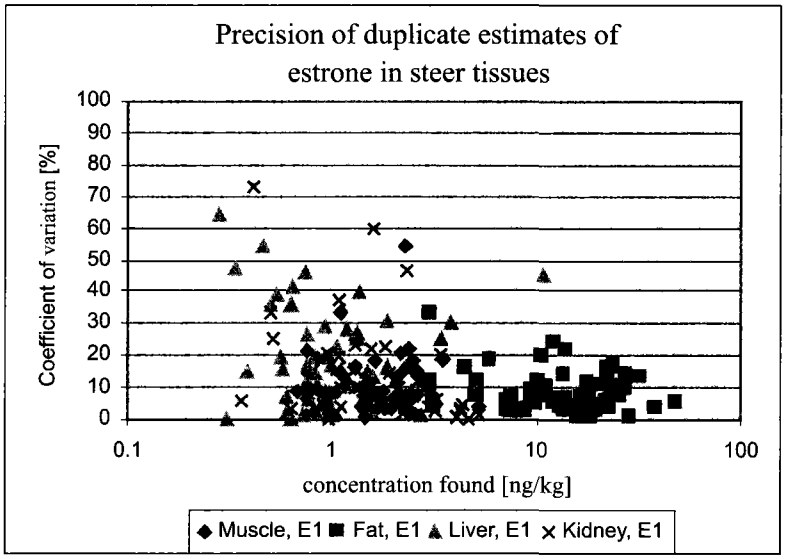


Figure 4c



The results obtained in a residue study with non-pregnant heifers were evaluated in the same way to estimate the precision of the method used. The results are shown in the following three Figures 5a - 5c for testosterone, estradiol-17β and estrone and the four edible tissues muscle, fat, liver and kidney

Figure 5. Precision of duplicate estimates of testosterone, estradiol-17β and estrone in heifer tissues

Figure 5a

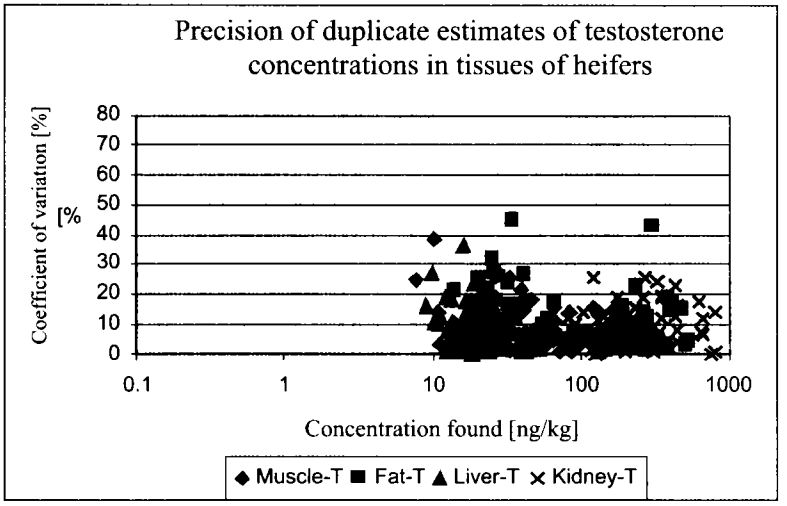


Figure 5b

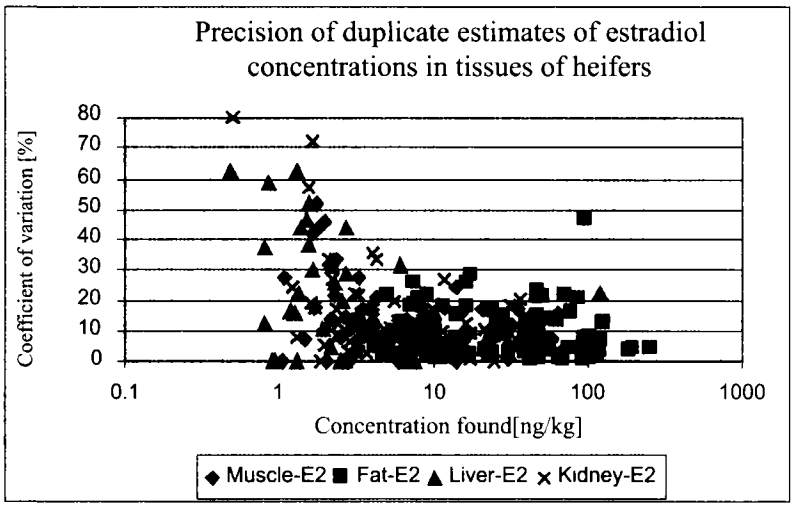
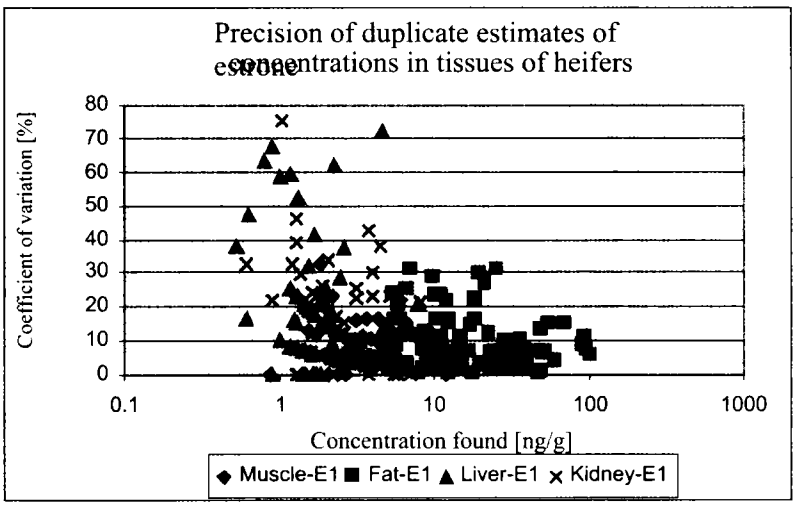


Figure 5c



Information on residues

Information obtained from studies of the analytical methodology

Some results of the method validation studies can be used as initial estimates of physiological levels of the three hormones in untreated steers. These data, obtained from six animals, are summarised in Table 9. The method was also used in a preliminary study to measure and to compare hormone concentrations in three pregnant heifers and in an untreated steer, as illustrated in Table 10.

Table 9. Hormone concentrations in tissues of six hormonally untreated steers

Analytical parameter	Concentration (ng/kg)					
	Estrone		Estradiol-17β		Progesterone	
	Muscle	Fat	Muscle	Fat	Muscle	Fat
Range	0.62-5.0	1.7-9.5	0.12-0.50	1.2-2.2	110-4200	530-7700
Mean	2.4	8.0	0.30	1.7	1570	2570
Standard Deviation	1.53	6.1	0.14	0.32	1650	2920
Geometric Mean	1.9	6.1	0.30	1.7	760	1530
Median	2.5	7.4	0.40	1.7	1080	970

Table 10. Hormone concentrations (ng/kg) in tissues of pregnant heifers

Days pregnant	Hormone concentrations (ng/kg) in tissues of pregnant heifers											
	Muscle			Fat			Kidney			Liver		
	E_1	E_2	P	E_1	E_2	P	E_1	E_2	P	E_1	E_2	P
188	110	14.6	6350	1920	55.6	318000	207	280	7620	10.4	81.7	3180
243	458	44.9	6160	4880	139	106000	500	417	4090	258	630	2170
266	638	42.6	17800	5330	131	294000	602	484	6860	584	1590	4890
	Hormone concentrations (ng/kg) in tissues of a steer											
	E_1	E_2	P	E_1	E_2	P	E_1	E_2	P	E_1	E_2	P
	1.8	0.97	624	10.2	1.4	3240	13	25.4	675	3.4	4.4	596

E_1 = Estrone, E_2 = Estradiol-17β, P = progesterone

Residue studies

Residues in steers implanted with SYNOVEX S

A residue study was conducted with 64 healthy, beef-type steers, weighing between 317.5 and 340.2 kg, which had not previously been implanted with any growth promoting substance (Study code IAS 1012-021.1.). The total duration of the study (minus acclimatisation period) was 120 days. Since all animals were slaughtered on day 120 and groups of animals were implanted on days 0, 30, 59, 90, 105, the time period between implantation and slaughter was 15, 30, 61, 90, and 120 days, respectively. Each treatment group initially comprised 8 animals and 21 animals served as a control. Some treatment groups consisted of 7 animals since two animals died and one developed chronic respiratory problems.

The statistical parameters describing the concentrations of hormones found in edible tissues were calculated from the individual results: number of animals sampled per control and treatment group, respectively, minimal concentration found, maximal concentration found, arithmetic mean and standard deviation, geometric mean and median. The results

of these calculations are summarised in Table 11 following the full discussion of residues. The means and standard deviations calculated from the raw data by the current reviewer sometimes slightly deviate from the results given in the original report. The current reviewer decided to uniformly present all data as results of duplicate estimates. The report of the authors of the studies use averages of a varying number of >2 of replicates if the same samples had been analysed more than twice and by different technicians (e.g., in order to determine the transferability of the method and to obtain an estimate of interlaboratory variability).

Residues in nonpregnant heifers implanted with SYNOVEX H

A study was conducted in which non-pregnant heifers were implanted with Synovex H Heifer finishing implant (study code IAS 1012-116.1.) (Kushinskyand Duffy, undated). The product contained 200 mg testosterone propionate and 20 mg estradiol benzoate and is recommended for use in heifers weighing 400 lbs. or more during the last 60 to 150 days of the fattening period. Animals were slaughtered 30, 60, 89 or 119 days after implantation and residues of estrone, estradiol, and testosterone were determined in fat, muscle, kidney and liver. Each sample was analysed in duplicate. Statistical parameters were calculated as described for the steer study. These data are given in Table 12 below.

Residues in pregnant heifers implanted with SYNOVEX H

It is stated in the dossier of the SYNOVEX implants (Kushinsky and Duffy, 1982) that pregnant heifers enter feedlots and are brought to slaughter in significant numbers and therefore consist of a significant part of the meat reaching the consuming public. A study was conducted in which tissues were obtained from pregnant heifers that were approximately 120, 180 or 240 days pregnant. Both the control animals the animals implanted 61, 90 or 120 days before slaughter were used. Fenprostalene was used for synchronisation of the estrous cycles to facilitate breeding of the animals by artificial insemination. Additional control animals, which were not synchronised, were also used. Statistical parameters were calculated as described for the steer study. These data are given in Table 13 below.

Residues in calves implanted with SYNOVEX

Thirty six suckling steer calves and 42 suckling heifer calves (purebred and crossbred Hereford) averaging 59.4 kg (range 34 to 94 kg) were implanted with Synovex-C implant, comprising 10 mg of estradiol benzoate plus 100 mg of progesterone ((Kushinsky and Duffy, 1983: Sheldon *et al.*, 1983). An equal number of animals served as non-implanted controls. Average daily growth rates prior to weaning were in the range of 0.59 to 0.64 kg/day. Calves were re-implanted 118 and 240 days later with steers receiving the Synovex S implant and heifers receiving the Synovex H implant. All implants were commercial products. The typical increase in average daily gain due to Synovex implants was apparent by 240 days.

Four to eight implanted and non-implanted calves of each sex were slaughtered at 61,119,241 301, 329 and 360 days after the initial implant was administered. Samples of edible tissues were analysed for concentrations of estradiol-17β, estrone and either progesterone or testosterone.

Generally, larger residues were found after each successive implantation. The residues of estrogens found after a single implantation were similar to those previously found for steers and heifers implanted with Synovex S and Synovex H, respectively (Study code IAS-1012-341.3). Results of residues in male calves are summarised in Tables 14 – 17, and residues in female calves are summarised in Tables 18 – 21.

Table 11. Concentrations (ng/kg) of Estrone, Estradiol-17β and Progesterone in edible tissues of steers implanted with SYNOVEX-S

Tissue	Day after implantation	Concentrations (ng/kg) of estrone, estradiol-17β and progesterone in edible tissues of control steers and steers implanted with SYNOVEX-S																	
		Estrone							Estradiol-17β						Progesterone					
		n	Min.	Max.	Mean	S.D.	Geo. Mean	Median	Min.	Max.	Mean	S.D.	Geo. Mean	Median	Min.	Max.	mean	S.D.	Geo. Mean	Median
Fat	Control	21	1.49	18.9	8.62	5.14	7.11	7.37	0.10	3.66	1.89	1.14	1.37	1.91	670	6280	2535	1762	2061	2290
	15	8	11.4	39.2	20.2	9.21	18.6	18.6	25.3	51.2	41.4	8.53	40.5	42.2	1430	4750	3196	1167	2979	3345
	30	8	3.04	25.8	14.4	7.71	12.1	14.7	5.13	61.5	28.6	21.8	20.8	26.0	1360	6400	3478	1641	3110	3725
	61	8	9.65	48.3	25.9	11.8	23.5	25.7	20.0	52.4	39.9	11.0	38.3	43.9	1680	5840	3528	1465	3260	3290
	90	7	13.2	27.8	18.0	5.43	17.4	16.3	12.2	48.3	26.8	11.7	24.7	25.6	1770	7710	4029	2444	3456	3350
	120	7	7.66	24.4	16.6	5.53	15.7	16.1	4.06	30.0	14.5	8.91	12.0	14.8	1490	4660	2657	1110	2482	2110
Muscle	Control	16	0.67	2.61	1.56	0.65	1.43	1.53	0.34	1.21	0.77	0.35	0.69	0.68	40	1410	271	327	188	150
	15	8	1.39	3.05	2.12	0.59	2.04	2.24	4.58	15.3	9.60	3.17	9.11	9.71	50	460	230	121	197	210
	30	8	0.75	3.51	1.98	0.85	1.81	2.04	1.00	15.3	8.27	5.14	6.01	8.74	160	310	227	52.5	222	220
	61	8	0.77	5.21	2.35	1.29	2.08	2.13	3.75	11.3	7.34	2.72	6.88	7.23	80	1650	419	507	282	260
	90	7	0.76	2.55	1.79	0.60	1.68	1.96	0.97	7.81	4.51	2.18	3.85	4.51	90	1670	439	573	250	180
	120	7	0.92	3.02	1.79	0.69	1.68	1.63	1.04	3.33	2.18	0.86	2.01	2.23	130	2460	583	836	342	270
Kidney	Control	18	0.18	2.34	1.03	0.50	0.90	1.02	0.55	2.78	1.7	0.6	1.55	1.53	10	530	161	141	111	135
	15	8	3.10	7.31	4.44	1.37	4.28	4.28	8.88	17.0	13.7	3.0	13.4	14	80	210	135	49	127	120
	30	8	1.20	4.39	2.55	1.23	2.30	2.18	1.42	17.6	9.45	5.4	7.6	9.7	70	150	114	31	110	120
	61	8	1.30	2.61	1.99	0.49	1.93	2.21	4.37	9.53	6.4	1.9	6.1	6.0	0	920	170	306	NC	80
	90	7	0.41	2.98	1.43	0.88	1.21	1.08	1.39	12.7	5.7	3.5	4.7	5.38	10	930	321	415	117	90
	120	7	0.63	2.56	1.70	0.64	1.57	1.56	2.37	6.39	4.2	1.3	4.0	3.91	60	540	173	172	128	90
Liver	Control	18	0.28	1.00	0.65	0.24	0.60	0.73	0.36	1.72	0.91	0.42	0.82	0.87	170	380	254	74	244	240
	15	8	1.32	3.85	2.04	0.84	1.91	1.73	1.92	8.05	5.36	2.04	4.93	5.51	130	240	175	34	172	175
	30	8	0.96	3.44	1.79	0.94	1.61	1.48	2.76	16.8	6.74	4.93	5.50	4.24	110	210	155	33	152	145
	61	8	0.54	10.9	2.09	3.57	1.11	0.79	0.7	17.4	4.51	5.48	2.83	2.42	280	490	349	68	343	340
	90	7	0.46	2.46	1.21	0.71	1.05	1.06	0.85	15.6	5.41	5.15	3.66	3.37	110	400	240	117	217	195
	120	7	0.50	0.93	0.72	0.19	0.70	0.64	0.69	2.07	1.39	0.42	1.32	1.4	180	370	272	81	262	245

n = number of specimens analysed; s.d. = standard deviation; geo. mean = geometric mean. NC = not calculated (see min. = zero)

Table 12. Concentrations (ng/kg) of estrone, estradiol-17β and testosterone in edible tisssues of heifers implanted with SYNOVEX H

Tissue	Day after implantation	Concentrations (ng/kg) of estrone, estradiol-17β and testosterone in edible tissues of control heifers and heifers implanted with SYNOVEX-H																	
		Estrone							Estradiol-17β						Testosterone					
		n	Min.	Max.	Mean	S.D.	Geo. Mean	Median	Min.	Max.	Mean	S.D.	Geo. Mean	Median	Min.	Max.	Mean	S.D.	Geo. Mean	Median
Fat	Control	35	2.11	31.7	10.9	5.94	9.54	10.4	3.93	71.5	13.4	14.1	10.1	9.19	14.5	44.7	25.9	6.94	25.1	23.9
	30	20	5.73	105	45.3	29.4	36.6	36.5	4.76	260	86.7	65.9	61.2	73.2	35.1	1113	359	228	274	290
	60	25	8.9	55.9	27.8	13.8	24.5	26.0	11.1	127	49.2	30.8	40.2	48.1	24.1	377	142	104	103	129
	89	10	14.8	70.2	35.3	17.5	31.7	29.0	10.5	118	62.3	34.9	51.3	54.3	26.6	203	115	69.8	91.2	132
	119	10	6.76	32.7	13.6	8.06	12.0	11.6	8.2	41.3	17.5	13.0	14.4	11.3	14.7	54.0	32.1	12.1	30.0	30.9
Muscle	Control	15	1.29	4.33	2.54	1.12	2.32	2.28	1.05	35.2	5.80	9.05	3.41	2.91	7.58	33.3	19.6	7.09	18.3	21.7
	30	20	0.88	12.8	6.42	3.68	5.28	5.27	2.14	65.8	33.2	19.6	24.8	30.5	11.3	183	102	48.1	85.6	101
	60	10	1.94	6.34	3.98	1.45	3.74	3.76	3.57	20.5	10.7	5.14	9.49	10.2	16.8	84.4	46.7	22.3	41.6	40.9
	89	10	2.92	12.3	6.40	3.38	5.67	4.77	2.39	16.8	10.1	4.44	8.96	10.4	20.9	135	58.7	34.2	50.6	51.9
	119	10	1 68	4.08	2.55	0.75	2.46	2.55	1.09	5.58	2.55	1.35	2.28	2.07	14.0	54.9	31.2	12.4	29.1	27.1
Kidney	Control	15	0.61	2.97	1.42	0.56	1.32	1.41	0.5	11	2.89	2.50	2.26	1.97	81	383	189	91.6	170	169
	30	10	2.15	9.7	5.87	2.27	5.45	5.37	4.82	42.5	23.6	11.9	20.2	23.0	161	793	451	201	407	431
	60	15	1.47	8.32	3.55	2.02	3.16	3.03	2.44	36.9	9.83	8.98	7.57	7.51	122	661	228	143	203	180
	89	10	2.35	6.55	4.23	1.49	3.99	3.96	2.42	16.4	8.88	4.83	7.62	7.72	106	816	371	267	292	256
	119	10	1.3	2.7	1.75	0.46	1.7	1.7	1.59	6.01	3.16	1.41	2.92	2.85	168	444	307	89.3	294	330
Liver	Control	10	0.63	4.57	1.7	1.15	1.44	1.39	0.48	3.89	1.54	1.12	1.23	1.00	9.98	15.5	12.9	1.96	12.8	13.3
	30	10	1.49	11.5	3.69	3.36	2.80	2.04	4.28	121	23.1	36.6	11.3	6.75	18.5	51.2	34.1	9.98	32.8	31.8
	60	10	0.53	2.66	1.49	0.76	1.30	1.44	0.8	7.62	3.21	2.36	2.51	2.25	8.85	19.5	15.7	3.34	15.3	16.3
	89	10	1.01	1.94	1.51	0.29	1.49	1.54	1.2	6.49	3.28	1.78	2.91	2.65	14	42 5	22.6	7.89	21.6	20.3
	119	10	0.89	1.68	1.33	0.22	1.31	1.35	0.93	2.21	1.48	0.42	1.43	1.36	10.8	26.9	16.1	5.26	15.5	14.9

n = number of specimens analysed; s.d = standard deviation; geo.mean = geometric mean

Table 13. Concentrations of estrone, estradiol-17β and testosterone in edible tissues of pregnant heifers implanted with SYNOVEX H

Treatment group	days pregnant	n	min.	max.	mean	s.d.	geom.	median	min.	max.	mean	s.d.	geom.	median	min.	max.	mean	s.d.	geom.	median
			Estrone in Fat (ng/kg)						Estradiol-17 β in Fat (ng/kg)						Testosterone in Fat (ng/kg)					
Unsynchronised control	120	7	77.8	1964	780	641	526	811	11.5	52.7	30.9	13.9	28.0	31.8	228	530	406	101	394	414
Synchronised controls	120	10	213	3175	1283	885	1008	1132	13.7	77.5	42.1	19.8	37.8	38.8	411	1008	590	176	570	540
61 Days implanted	120	5	44.5	877	421	352	273	300	43.8	140	82.9	37.6	76.3	79.6	446	942	751	198	727	803
Synchronised controls	180	11	983	4579	2717	1259	2417	2737	30.6	159	71.5	37.2	64.0	65.8	511	1000	751	174	732	731
61 Days implanted	180	5	578	3448	1896	1290	1533	1299	62.7	208	123	58.9	112	136	701	1463	1047	274	1018	1012
Synchronised controls	240	4	1037	4663	2786	1497	2441	2723	23.7	108	67.5	34.6	59.1	69.0	445	1002	694	231	666	666
61 Days implanted	240	4	2637	5980	4614	1513	4399	4920	95.4	152	136	27.2	134	149	981	1318	1195	158	1186	1240
			Estrone in Muscle (ng/kg)						Estradiol-17 β in Muscle (ng/kg)						Testosterone in Muscle (ng/kg)					
Unsynchronised control	120	8	13.9	462	203	170	127	154	4.3	31.6	15.6	11.6	11.9	11.3	126	547	302	163	266	247
Synchronised controls	120	10	37.7	299	156	79.3	133	173	5.12	21.3	13.2	5.21	12.2	12.8	174	500	267	101	252	230
61 Days implanted	120	5	22.5	178	83.1	67.8	60.8	60.0	21	44.3	30.6	11.2	29.1	24.4	189	542	357	130	337	329
Synchronised controls	180	11	85.0	1115	482	301	387	422	9.82	57.8	27.3	14.3	24.1	24.0	232	557	343	117	327	312
61 Days implanted	180	5	52.2	285	167	108	137	122	13.7	35.0	24.7	7.88	23.6	23.4	291	479	356	81.4	349	309
Synchronised controls	240	4	208	774	523	240	470	555	9.54	45.8	32.7	16.1	28.0	37.8	202	630	418	107	386	421
61 Days implanted	240	4	196	435	353	109	338	391	15.0	33	26.3	8.16	25.2	28.7	251	457	370	89.0	361	387
			Estrone in Liver (ng/kg)						Estradiol-17 β in Liver (ng/kg)						Testosterone in Liver (ng/kg)					
Unsynchronised control	120	8	1.3	94.3	29.7	33.0	13.1	18.5	3.99	139	58.4	49.9	34.3	49.9	20.9	56.8	38.9	13.2	36.9	36.7
Synchronised controls	120	10	6.5	53.2	18.2	15.1	14.2	12.1	26.7	271	82.5	75.9	61.17	53.1	40.9	73.3	52.8	10.1	52.0	50.3
61 Days implanted	120	5	3.01	7.84	5.52	2.19	5.141	6.03	10.4	27.7	18.7	7.38	17.6	16.0	28.9	46.2	37.6	6.94	37.1	39.4
Synchronised controls	180	11	32.5	320	115	82.6	94.1	91	73	925	380	280	299	242	99.7	147	121	19.4	120	117
61 Days implanted	180	5	8.95	44.7	23.4	13.7	20.3	19.1	22.7	100	50.4	33.6	42.4	34.7	49.7	71.65	60.6	7.96	60.2	60
Synchronised controls	240	4	66.3	196	145	55.4	135	159	526	1396	1027	365	967	1094	207	353	273	70.1	266	266
61 days implanted	240	4	8.52	95.8	34.5	41.5	20.8	16.8	50.7	438	198	173	145	152	81.9	102	90.2	8.75	89.9	88.6
			Estrone in Kidney (ng/kg)						Estradiol-17β in Kidney (ng/kg)						Testosterone in Kidney (ng/kg)					
Unsynchronised control	120	5	49.4	101	84.2	21.0	81.6	87.1	63.8	191	127	46.3	119	120	1371	2373	1930	453	1885	2086
Synchronised controls	120	10	31.1	268	85.3	69.4	69.9	60.7	68.8	258	118	58.6	108	99.6	1185	2250	1513	331	1484	1413
61 Days implanted	120	5	11.8	66.6	29.7	23.1	23.8	17.4	30.9	138	62.3	44.8	52.3	43.2	1276	2344	1856	426	1814	1808
Synchronised controls	180	6	37.5	304	166	94.0	139	151.3	90.9	354	230	99.9	208	259	2010	5644	3505	1537	3248	2805
61 Days implanted	180	5	9.35	110	59.4	36.6	45.9	64.7	39.4	184	126	58.2	111	140	1579	2813	1974	510.3	1927	1751
Synchronised controls	240	4	86.2	186	142	41.2	137	148	163	352	274	84.8	263	291	1632	6801	4014	2269	3503	3812
61 Days implanted	240	4	117	271	214	67	204	234	165	407	318	107	301	351	1782	4311	2914	1057	2773	2782

Table 14. Hormone concentrations (ng/kg) in muscle of calves (steers) implanted with SYNOVEX C and S

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A	5	1.35	4.36	2.37	1.18	2.18	2.05	0.56	1.47	0.94	0.36	0.78	0.78	2.63
E2		5	10.7	12.2	11.5	0.58	11.4	11.7	0.72	1.44	1.11	0.30	1.08	1.01	11.5
P		5	134	1336	764	463	597	863	49.1	548	292	191	223	318	2.72
E1	B	5	1.36	3.53	2.17	0.93	2.02	1.88	0.32	1.09	0.57	0.31	0.55	0.49	3.84
E2		5	3.75	6.87	5.67	1.23	5.54	5.84	0.36	0.73	0.51	0.14	0.50	0.47	12.4
P		5	128	602	313	199	265	242	121	860	404	325	298	302	0.8
E1	C	5	1.32	4.08	2.72	1.10	2.52	2.78	1.13	2.31	1.73	0.55	2.31	1.93	1.44
E2		5	2.78	13.4	7.33	4.38	6.29	5.52	1.89	2.72	2.21	0.32	2.20	2.18	2.53
P		5	328	804	517	239	476	370	356	946	563	226	532	507	0.73
E1	D	5	2.82	6.68	4.42	1.57	4.2	4.53	0.63	1.4	0.93	0.34	1.18	0.79	5.73
E2		5	15.1	71.4	29.6	23.5	24.7	21.75	0.75	8.79	2.91	3.36	1.89	1.3	16.7
P		5	297	414	355	43.3	352	353	148	2887	886	1129	528	438	0.81
E1	E	5	1.63	3.77	2.77	0.88	2.66	2.53	1.28	2.41	1.95	0.48	1.28	2.22	1.14
E2		5	6.9	14.8	9.72	2.97	9.41	9.06	0.81	1.84	1.23	0.43	1.17	1.02	8.88
P		5	275	1335	541	452	440	331	305	1009	607	279	557	544	0.61
E1	F	4	1.08	6.01	3.29	2.53	2.48	3.04	0.69	3.55	1.61	1.20	0.72	1.14	2.67
E2		4	3.77	9.78	6.23	2.60	5.85	5.68	0.52	3.64	1.69	1.44	1.22	0.97	5.86
P		4	357	1461	777	477	681	645	1339	3642	2413	889	2277	2511	0.26

Table 15. Hormone concentrations (ng/kg) in fat of calves (steers) implanted with SYNOVEX C and S

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A	5	16.3	36.3	22.0	8.59	20.8	16.9	1.66	8.85	5.36	3.15	6.37	6.37	2.65
E2		5	35.3	47.6	38.6	5.14	38.3	36.2	2.85	4.39	3.3	0.64	3.3	3.1	11.7
P		5	3860	15500	8616	4938	7545	6200	2630	12650	7340	3686	6502	7780	0.80
E1	B	5	2.37	36.9	16.8	13.7	11.5	15.9	0.97	19.5	6.10	7.73	2.63	2.63	6.0
E2		5	2.37	35.6	24.0	14.2	17.2	30.1	1.92	22.9	6.73	9.03	4.08	3	10.0
P		5	5030	12720	8192	2924	7799	7840	3550	1.41e5	34230	59775	12478	8720	0.90
E1	C	5	5.02	27.1	14.7	10.0	11.8	11.4	2.92	7.25	4.83	1.99	7.25	3.85	2.96
E2		5	7.59	43.3	24.8	16.8	19.7	21.15	2.38	4.74	3.28	1.07	3.15	2.6	8.13
P		5	6910	41750	16458	14402	13051	11900	3350	64600	19244	25771	10375	9780	1.22
E1	D	5	22.2	61.4	40.0	14.7	37.8	38.5	1.93	5.2	3.09	1.40	2.11	2.35	16.4
E2		5	34.8	64.2	52.6	12.8	51.2	57.0	1.68	4.52	2.88	1.12	2.71	2.7	21.1
P		5	4030	10390	7924	2526	7530	7980	2350	7670	5234	2317	4774	5150	1.55
E1	E	5	10.7	35.2	19.8	9.4	18.2	18.7	2.47	7.77	5.07	1.98	4.15	4.96	4.06
E2		5	18.0	58.6	32.6	17.0	29.5	22.9	2.56	4.71	3.16	0.90	3.07	2.75	8.32
P		5	4890	8950	6504	1676	6342	5690	3290	8450	6383	2386	5986	6895	0.83
E1	F	4	6.44	23.3	15.6	8.01	13.8	16.42	1.9	7.09	3.64	2.104	1.9	3.1	5.30
E2		4	14.1	27.9	19.4	6.38	18.7	17.88	2.87	10.8	5.81	3.4	5.08	4.59	3.89
P		4	3600	11240	6638	3536	5967	5855	4900	16400	9008	4647	8181	6680	0.88

A = treatment group: implanted day slaughtered day 61 **B** = treatment group: implanted day 0 slaughtered day 119
C = treatment group: implanted day 0 implanted day 118 slaughtered day 241
D = treatment group: implanted day 0 implanted day 118 implanted day 240 slaughtered day 301
E = treatment group: implanted day 0 implanted day 118 implanted day 240 slaughtered day 329
F = treatment group: implanted day 0 implanted day 118 implanted day 240 slaughtered day 360
Testosterone was not measured. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

Table 16. Hormone concentrations (ng/kg) in liver of calves (steers) implanted with SYNOVEX C and S

Analyte	Treatment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A	5	1.87	2.99	2.43	0.45	2.39	2.38	1.58	2.22	1.78	0.27	1.58	1.66	1.43
E2		5	2.04	3.65	2.91	0.65	2.85	3.07	1.05	3.64	1.78	1.05	1.61	1.43	2.15
P		5	61.9	100	74.4	15.1	73.3	68.4	47.7	113	68.1	25.7	65.0	59.95	1.14
E1	B	5	0.62	1.51	1.15	0.39	1.09	1.22	0.88	1.23	1.06	0.16	1.12	1.12	1.09
E2		5	1.86	2.62	2.05	0.32	2.03	1.93	1.31	1.97	1.57	0.26	1.55	1.56	1.24
P		5	136	161	142	10.3	142	139	74.0	133	104	23.8	102	111	1.25
E1	C	5	1.17	2.62	1.76	0.59	1.68	1.61	1.13	2.49	1.69	0.54	1.65	1.65	0.98
E2		5	2.99	6.32	4.23	1.31	4.08	3.99	2.43	4.38	3.45	0.79	3.37	3.24	1.23
P		5	131	239	166	44.3	161	158	72.6	115	93.3	19.4	91.6	102.5	1.54
E1	D	5	1.18	7.16	2.72	2.54	2.09	1.38	0.9	2.26	1.27	0.57	1.2	1.03	1.34
E2		5	2.88	28.2	8.69	10.9	5.57	4	1.75	3.39	2.48	0.67	2.40	2.66	1.50
P		5	125	320	225	78.1	213	247	110	189	151	30.8	149	147	1.68
E1	E	5	1.51	6.66	2.88	2.13	2.46	2.07	0.97	1.74	1.26	0.31	0.97	1.19	1.74
E2		5	3.03	8.03	4.90	1.90	4.64	4.32	1.66	1.89	1.77	0.09	1.77	1.76	2.45
P		5	144	233	201	35.1	198	206	113	191	147	31.8	142	133	1.55
E1	F	4	2.06	2.57	2.37	0.27	2.36	2.49	1.43	1.96	1.72	0.20	1.43	1.76	1.41
E2		4	3.06	5.31	4.28	1.14	4.17	4.47	2.9	4.14	3.62	0.45	3.6	3.71	1.20
P		4	150	207	185	30.3	183	197	116	164	128	22.1	130	128	1.60

Table 17. Hormone concentrations (ng/kg) in kidney of calves (steers) implanted with SYNOVEX C and S

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A	5	2.96	4.36	3.67	0.51	3.64	3.79	0.7	1.7	1.05	0.40	0.7	0.92	4.12
E2		5	5.63	7.28	6.54	0.61	6.52	6.64	0.28	2.45	1.46	0.8	1.19	1.36	4.88
P		5	168	1473	777	522	609	700	182	807	375	265	315	217	3.22
E1	B	5	1.87	3.32	2.66	0.55	2.61	2.75	0.68	1.78	1.03	0.45	1.02	0.97	2.84
E2		5	2.7	6.81	4.98	1.62	4.74	4.78	0.82	2.73	1.28	0.82	1.14	0.91	5.25
P		5	121	609	353	222	296	246.5	135	1264	481	460	349	286	0.86
E1	C	5	3.43	4.81	4.21	0.52	4.18	4.33	2.26	3.53	2.92	0.48	2.26	2.8	1.55
E2		5	2.55	10.0	5.65	3.15	4.91	6.36	1.36	3.76	2.09	0.97	1.95	1.62	3.92
P		5	297	515	388	94.4	379	344	213	900	453	264	403	361	0.95
E1	D	5	4.45	7.81	5.73	1.51	5.58	4.99	0.64	2.5	1.20	0.77	0.64	0.89	5.61
E2		5	13.9	24.9	17.8	4.39	17.4	17.45	2.39	16.8	5.64	6.25	4.04	2.9	6.02
P		5	170	295	225	57.5	219	209	79.7	2402	733	951	396	359	0.58
E1	E	5	1.9	6.09	4.27	1.63	3.96	4.94	1.17	3.57	2.41	0.98	3.13	2.42	2.04
E2		5	5.94	12.2	8.41	2.54	8.12	7.49	1.91	7.81	3.29	2.54	2.77	2.09	3.58
P		5	126	1047	358	389	255	199	203	803	387	239	342	289	0.69
E1	F	4	2.51	4.84	3.91	1.02	3.79	4.135	2.26	3.39	2.55	0.48	2.26	2.38	1.74
E2		4	4.73	8.4	6.82	1.57	6.67	7.08	1.87	4.33	2.77	0.92	2.67	2.62	2.70
P		4	327	792	492	206	464	424	619	2303	1336	667	1200	1456	0.29

A = treatment group: implanted day 0, slaughtered day 61 **B** = treatment group: implanted day 0, slaughtered day 119.
C = treatment group: implanted day 0, implanted day 118, slaughtered day 241
D = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 301
E = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 329
F = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 360
Testosterone was not measured. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

Table 18. Hormone concentrations (ng/kg) in muscle of calves (heifers) implanted with SYNOVEX C and H

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A*	5	1.71	2.52	2.08	0.35	2.06	2.07	0.66	1.15	0.91	0.20	1.06	0.89	2.33
E2		5	6.15	22.9	13.0	6.14	11.9	12.3	0.96	1.91	1.42	0.39	1.37	1.51	8.11
P		5	311	1489	698	487	587	447	109	916	435	392	298	182	2.46
E1	B*	5	2.74	4.6	3.84	0.71	3.78	4	2.25	5.25	3.07	1.26	2.25	2.45	1.63
E2		5	3.23	14.1	7.32	4.38	6.38	5.57	0.8	1.43	1.14	0.24	1.12	1.16	4.80
P		5	209	744	423	215	380	453	96.1	1089	540	373	412	581	0.78
E1	C**	5	2.49	4.04	3.33	0.60	3.28	3.48	2.36	3.31	2.61	0 40	2.38	2.49	1.40
E2		5	6.17	11.7	8.42	2.25	8.19	7.86	2.15	9.01	3.68	2.99	3.06	2.41	3.26
T		5	29.7	53.6	38.7	10.1	37.7	33.6	15	22.1	17.5	2.75	17.3	16.8	2
E1	D**	5	3.31	15.0	6.96	4.97	5.80	4.36	1.29	2.28	1.68	0.41	1.94	1.48	2.95
E2		5	13.6	97.9	38.0	34.5	29.3	23.05	1.38	6.37	3.42	2.03	2.95	2.74	8.41
T		5	77.1	546	196	197	147	115	18.2	52.1	37.4	12.4	35.4	38.2	3.01
E1	E**	5	2.05	4.08	2.60	0.87	2.50	2.08	0.9	1.77	1.39	0.33	1.49	1.49	1.40
E2		5	2.64	17.6	9.23	5.38	7.84	9.21	1.19	7.01	2.97	2.35	2.42	2.03	4.54
T		5	13.2	66.6	43.1	20.1	39.6	52.3	14.2	27.2	21.3	5.61	20.6	21.7	2.41
E1	F**	5	0.65	3.32	1.88	1.17	1.54	2.01	1.35	3.46	2.91	0.89	3.46	3.35	0.6
E2		5	1.2	10.7	3.67	3.98	2.6	2.06	1.93	17.3	9.51	6.03	7.54	8.1	0.25
T		5	23.4	75.7	47.0	19.2	43.8	46.1	38.9	90.2	64.5	20.2	61.9	59.2	0.78

Table 19. Hormone concentrations (ng/kg) in fat of calves (heifers) implanted with SYNOVEX C and H

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A*	5	16.9	23.9	20.2	3.08	20	20.1	2.24	6.11	3.88	1.87	6.11	3	6.7
E2		5	24.5	68.5	41.2	16.6	38.8	39.1	1.58	3.91	2.43	0.92	2.31	2.39	16.3
P		5	5180	14450	8554	3706	7992	6740	2090	12750	5624	4141	4681	4820	1.40
E1	B*	5	15.6	52.7	30.7	13.8	28.3	28.15	1.25	6.19	3.72	2.2	6.19	3.23	8.72
E2		5	11.0	86.5	38.5	30.6	29.7	28.1	2.25	4.31	2.944	0.84	2.86	2.88	9.76
P		5	5710	14250	8510	3445	8043	6760	6520	24800	12974	6989	11730	11950	0.57
E1	C**	5	8.9	25.0	18.4	6.47	17.3	20.2	2.59	24.7	9.92	8.94	24.7	7.07	2.86
E2		5	14.6	45.1	30.2	12.1	28.1	27.3	4.52	7.99	5.58	1.38	5.46	5.25	5.2
T		5	87.2	159	126	31.5	123	119	23.7	43.2	30.3	7.73	29.6	27.6	4.30
E1	D**	5	18.5	98.7	47.0	34.5	37.9	30	3.11	7.02	5.28	1.58	6.18	5.85	5.13
E2		5	27	141	74.5	42.8	64.6	75.7	3.25	10.03	6.19	3.38	5.465	4.77	15.9
T		5	162	552	337	173	301	280	20.1	136	69.4	49.4	54.9	52.3	5.35
E1	E**	5	13.0	28.9	19.6	6.30	18.9	17.0	4.86	11.8	7.88	2.51	6.87	7.73	2.19
E2		5	21.4	70.8	38.0	19.0	34.9	32.4	3.56	24.7	9.19	9.04	6.70	4.32	7.49
T		5	84.1	316	203	91.1	185	193	52.4	103	81.7	22.3	79.1	86.2	2.24
E1	F**	5	17.9	43.2	27.8	9.52	26.6	27.7	7.71	20.8	13.9	4.85	20.8	13.5	2.05
E2		5	24.7	63.25	41.9	16.1	39.3	44	4.58	7.28	5.36	1.11	5.28	4.94	8.91
T		5	171	329	216	64.3	209	194.5	19.1	72.4	46.1	19.4	42.3	46.7	4.17

A = treatment group: implanted day 0, slaughtered day 61 **B** = treatment group: implanted day 0, slaughtered day 119.
C = treatment group: implanted day 0, implanted day 118, slaughtered day 241
D = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 301
E = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 329
F = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 360
* =Testosterone not measured ** = progesterone not measured. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

Table 20. Hormone concentrations (ng/kg) in liver of calves (heifers) implanted with SYNOVEX C and H

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S.D.	Geo-Mean	Median	
E1	A*	5	0.64	3.02	1.45	0.93	1.26	1.07	0.55	0.8	0.64	0.10	0.62	0.62	1.73
E2		5	1.52	11.6	4.46	4.12	3.33	3.63	1.16	1.73	1.43	0.22	1.42	1.48	2.45
P		5	84.3	174	117	41.8	111	88.7	63.4	103	79.9	18.8	78.2	72.7	1.22
E1	B*	5	1.21	4.22	2.27	1.15	2.08	2.04	0.59	1.73	1.30	0.52	0.59	1.61	1.27
E2		5	1.55	5.46	3.46	1.53	3.17	3.58	1.19	2.11	1.71	0.33	1.69	1.8	1.99
P		5	127	298	227	77.2	215	274	153	271	196	44.3	193	188	1.46
E1	C**	5	1.15	2.2	1.60	0.4	1.56	1.63	1.1	1.38	1.22	0.138	1.11	1.15	1.42
E2		5	2.58	5.13	3.92	1.07	3.80	3.56	1.66	5.12	3.15	1.45	2.88	3.1	1.15
T		5	20.9	35.4	29.8	6.44	29.2	32.15	15.3	26.3	19.9	4.08	19.6	19.15	1.68
E1	D**	5	2.08	7	3.44	2.05	3.08	2.89	1.66	2.28	1.88	0.26	1.75	1.75	1.65
E2		5	7.82	33.4	15.0	10.6	12.8	12.6	7.08	8.22	7.56	0.41	7.56	7.51	1.68
T		5	21.6	35.9	28.6	5.26	28.2	28.7	14.1	19.6	17.6	2.17	17.5	18	1.59
E1	E**	5	0.69	1.43	1.12	0.28	1.09	1.22	0.81	1.19	1.02	0.155	0.94	1.03	1.18
E2		5	1.69	4.16	2.84	0.90	2.72	2.63	1.62	2.92	2.38	0.49	2.34	2.45	1.07
T		5	18.1	33.7	24.1	6.45	23.5	24	16.9	25.1	19.4	3.35	19.2	17.8	1.35
E1	F**	5	1.38	3.37	2.34	0.96	2.19	1.9	1.33	2.2	1.67	0.33	2.2	1.62	1.17
E2		5	3.19	6.69	4.90	1.61	4.68	5.26	1.26	2.65	1.91	0.51	1.86	1.83	2.87
T		5	43.2	57.6	47.6	5.74	47.3	46	21.15	42.6	33.3	8.51	32.3	35.5	1.30

Table 21. Hormone concentrations (ng/kg) in kidney of calves (heifers) implanted with SYNOVEX C and H

Analyte	Treat-ment	n	Statistics of treatment groups						Statistics of control groups						Ratio: implant/ control
			Min.	Max.	Mean	S.D.	Geo-Mean	Median	Min.	Max.	Mean	S D.	Geo-Mean	Median	
E1	A*	5	1.64	4.35	3.04	1.01	2.89	3.17	0.63	1.08	0.83	0.179	0.92	0.82	3.87
E2		5	5.1	13.4	7.86	3.21	7.43	6.9	0.99	1.99	1.38	0.39	1.34	1.22	5.66
P		5	249	993	548	296	487	485	112	800	399	295	310	288	1.68
E1	B*	5	2.66	5.11	3.65	1.07	3.53	3.1	1.38	2.26	1.67	0.36	1.38	1.5	2.07
E2		5	3.09	15.3	7.56	4.87	6.45	5.85	1.25	2.69	1.96	0.52	1.90	1.98	2.95
P		5	189	1371	533	481	411	373	143	1080	558	406	409	684	0.54
E1	C**	5	3.24	5.47	4.38	0.98	4.29	3.99	2.5	3.45	2.91	0.44	2.85	2.85	1.4
E2		5	6.11	13.6	9.61	3.04	9.22	9.4	2.19	3.7	2.76	0.59	2.72	2.61	3.60
T		5	142	445	249	122	228	214	125	255	199	59.3	191	214	1.00
E1	D**	5	4.35	10.1	6.52	2.15	6.27	5.83	1.98	5.27	2.94	1.36	1.98	2.45	2.38
E2		5	14.6	28.3	20.7	5.42	20.2	18.7	5.72	8.54	7.13	1.07	7.06	6.85	2.73
T		5	356	746	521	158	503	488	216	417	358	82.7	349	391	1.25
E1	E**	5	1.28	5.39	2.94	1.56	2.63	2.69	1.06	5.9	2.68	1.94	1.6	1.83	1.47
E2		5	2.24	15.6	7.55	5.41	5.95	7.35	1.53	5.15	2.46	1.53	2.19	1.83	4.02
T		5	136	464	299	133	274	276	217	686	391	189	358	390	0.71
E1	F**	5	1.97	6.06	3.18	1.65	2.92	2.75	0.7	1.64	1.08	0.43	1.64	0.86	3.20
E2		5	6.54	16.5	9.71	3.88	9.21	8.47	1.64	2.43	2.02	0.32	2.00	1.93	4.39
T		5	278	1033	489	313	430	396	178	804	508	232	454	552	0.72

A = treatment group: implanted day 0, slaughtered day 61 **B** = treatment group: implanted day 0, slaughtered day 119.
C = treatment group: implanted day 0, implanted day 118, slaughtered day 241
D = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 301
E = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 329
F = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 360
* =Testosterone not measured ** = progesterone not measured . n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

Studies in support of STEER-oid™

A 1982 tissue residue study was submitted to FDA under NADA 110-315. The study includes:

- data on the validation of radioimmunoassay
- results of tissue analyses conducted with muscle and fat samples obtained from 8 untreated steers and from 8 steers treated with STEER-oid™ (20 mg estradiol benzoate and 200 mg progesterone).

The investigators found no statistically significant differences between control animals and animals treated for 15 and 30 days with STEER-oid implants.

Analytical method

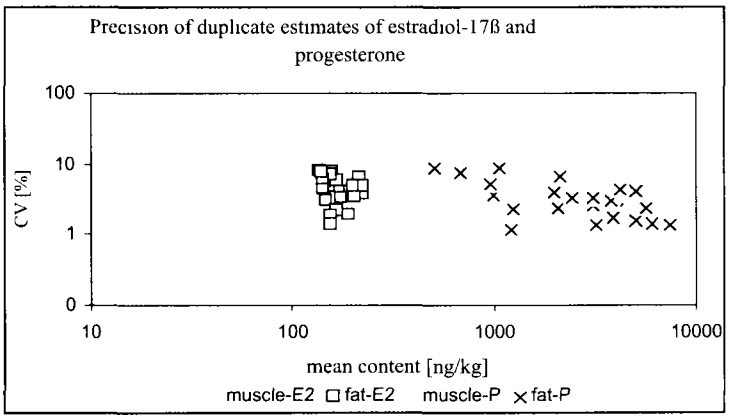
The results of the validation of the fully described analytical procedure (Marcus *et al.*, undated) are shown in Table 22. The sensitivity was sufficient to measure a difference of analytes as shown in Table 22. Recoveries averaged 77% in both muscle and fat. Recoveries were determined individually for every sample analysed. All results were corrected for recovery. The precision of the method was also tested. All samples were run in duplicate. The Coefficients of Variation (%CV) of duplicate estimates of the tissue hormone concentrations of the animals used in the study are shown in Table 22. . All samples were run in duplicate. The precision of the estimates was slightly dependent on the concentration of the analyte. This is shown in Figure 6. Cross-reactivity of other substances in the assay was also determined. There was a 0.6% cross-reactivity of Δ5-pregnenolone in the progesterone assay. Estradiol-17α (35%), estrone (32%) and estratriol (6%) all demonstrated some cross-reactivity in the estradiol-17ß assay. According to the authors, estradiol-17α is the only cross-reactive steroid that would elute with estradiol-17β during column chromatography of the crude extracts. The procedure has not been developed to include conjugates of the hormones in the determinations.

Table 22. Performance characteristics of the analytical method used to determine concentrations of hormones in steers treated with STEER-oid

Matrix	**Muscle**				**Fat**			
	Sensitivity		%CV (n=24)		Sensitivity		%CV (n=24)	
Analyte	LOQ (ng/kg)	difference* (ng/kg)	median	95th percentile	LOQ (ng/kg)	difference* (ng/kg)	median	95th percentile
Progesterone	430	500	5.7	15.0	1930	500	3.1	8.3
Estradiol-17ß	26	5	5.9	10.3	109	10	4.5	7.5

* = 'difference' defines the difference in the concentration of the analyte in two separate tissue samples that can be reliably differentiated and quantified.

Figure 6. Precision duplicate estimates of estradiol-17β and progesterone



Residues in tissues

Concentration of hormones in control steers and in steers implanted with STEER-oid are given in Tables 23 and 24 showing the percentage increase in hormone concentrations due to treatment. Figure 7 shows the results of the residue study. Since conjugates were not measured and residue data for liver and kidney were not obtained the study probably significantly underestimates the concentrations of the hormones in edible tissues.

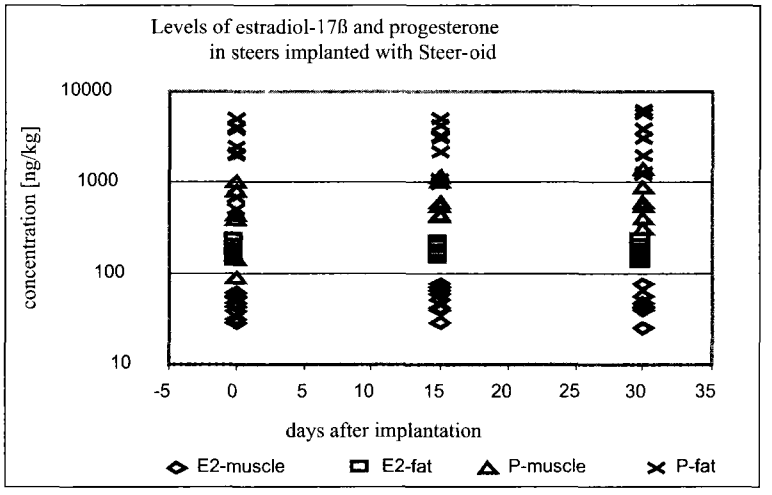
Table 23. Concentration of hormones in steers implanted with STEER-oid

Tissue	Analyte	Days after implantation	n	Hormone concentration (ng/kg)					
				Range		Mean	S.D.	Geometric Mean	Median
				Min.	Max.				
Muscle	Progesterone	Control	8	90	1025	564	347	430	620
		15	8	415	1160	799	326	737	820
		30	8	315	1380	769	370	689	742
Fat		Control	8	500	5045	2568	1616	2004	2265
		15	8	955	5055	2586	1569	2144	2585
		30	8	1210	7510	3816	2371	3117	3458
Muscle	Estradiol-17β	Control	8	28.5	61.0	44.5	11.7	43.1	44.3
		15	8	29.0	76.0	53.6	16.2	51.3	55.3
		30	8	25.5	77.0	46.2	15.1	44.2	41.8
Fat		Control	8	137.5	224.0	166.8	29.9	164.7	154.0
		15	8	143.5	217.5	172.3	28.7	170.3	161.0
		30	8	131.0	225.5	165.2	32.2	162.7	156.8

Table 24. Percentage increase in hormone concentrations in steers due to treatment

Tissue	Analyte	Days after implantation	Basis for the calculations		
			Arithmetic Mean	Geometric Mean	Median
Muscle	Progesterone	15	42	71	32
		30	36	60	20
Fat		15	0.7	7	14
		30	49	56	53
Muscle	Estradiol-17β	15	21	19	25
		30	3.8	2.5	-5.6
Fat		15	3.3	3.4	4.5
		30	-1.0	-1.2	1.8

Figure 7. Estradiol-17β and progesterone levels in steers implanted with STEER-oid



Studies in support of the HEIFER-oid™ implant

A 1983 tissue residue study was submitted to FDA under NADA 135-906 was evaluated. The study includes:

- data on the validation of a fully described radioimmunoassay procedures for testosterone and estradiol, respectively, in muscle and fat,
- results of tissue analyses conducted with muscle and fat samples obtained from 8 untreated heifers and from 8 heifers treated by ear implant with HEIFER-oid™ (200 mg testosterone propionate and 20 mg estradiol-benzoate) for 15 and 30 days, respectively (Marcus, undated).

Analytical method

The assays were validated with respect to sensitivity and recovery, linearity and parallelism of the calibration curve, and cross-reactivity with other steroids.

The sensitivity was sufficient to measure a difference of between 5 and 20 ng/kg at level between 37 and 274 ng/kg, as shown in Table 25. Recoveries of testosterone and estradiol were not significantly different, averaging about 74% in muscle and fat. Excellent linearity (logit B/B_0 vs. log dose) was demonstrated throughout the experimental working range. Concentrations found in serial dilutions of sample extracts paralleled the calibration curve. Cross-reactivity was tested with 17 steroids. The substances exhibiting the strongest cross-reactivities in the testosterone assay were Δ4-androstanediol (0.9%), 5α-androstan-3β,17β-diol (3%) and dihydrotestosterone (66%). The cross-reactivity of dihydrotestosterone was minimised through chromatographic separation on celite columns. Estradiol-17α (6%), estratriol (0.4%) and testosterone (0.3%) all demonstrated some cross-reactivity in the estradiol-17ß assay. According to the authors, estradiol-17α is the only cross-reactive steroid that would elute with estradiol-17β during column chromatography of the crude extracts. The procedure has not been developed to include conjugates of the hormones in the determinations.

Recovery was estimated with ^{3}H-labeled steroids. All results were corrected for recovery. All samples were run in duplicate. The coefficient of variation of duplicate estimates of the tissue hormone concentrations of the animals used in the study is shown in Table 25. The method was not designed to include conjugates of the hormones in the determinations of residues.

Table 25. Performance characteristics of the analytical method used to determine concentrations of hormones in steers treated with Steer-oid

Matrix:	**Muscle**				**Fat**			
	Sensitivity		%CV (n=24)		Sensitivity		%CV (n=24)	
Analyte	LOQ (ng/kg)	Difference* (ng/kg)	Median	95th Percentile	LOQ (ng/kg)	Difference* (ng/kg)	Median	95th Percentile
Testosterone	104	5	3.9	9.8	274	10-20	3.5	8.7
Estradiol-17ß	37	5	6.6	19.0	154	10	4.8	11.9

* = 'difference' defines the difference in the concentration of the analyte in two separate tissue samples that can be reliably differentiated and quantified

Residues in tissues

The investigators concluded that small but statistically insignificant differences were observed between 8 untreated heifers and 8 treated by ear implant with Heifer-oid for 15 days and 8 others treated for 30 days with the same product. Statistical parameters are given in the below Table 26.

The increases in the concentrations of the two hormones were in fact very low. However, since conjugates were not measured and residue data for liver and kidney were not obtained, the study probably significantly underestimates the concentrations of the hormones in edible tissues.

Table 26. Concentration of hormones in control animals and in steers implanted with the HEIFER-oid™ implant

Tissue	Analyte	Days after implantation	n	Hormone concentration (ng/kg)					
				Range		Mean	S.D.	Geometric Mean	Median
				Min.	Max.				
Muscle	Testosterone	Control	8	56.5	186.5	95.6	25.1	92.4	102.0
		15	8	129.5	311.5	108.3	20.9	106.3	114.3
		30	8	71.5	203.5	106.4	22.6	103.9	107.8
Fat		Control	8	132.5	377.5	246.7	46.3	242.9	238.3
		15	8	61.5	224.0	277.4	55.3	272.7	272.0
		30	8	139.5	333.0	271.3	44.1	268.2	258.3
Muscle	Estradiol-17β	Control	8	13.5	58.5	34.9	15.8	31.3	37.5
		15	8	10.5	71.0	39.8	19.7	34.5	42.0
		30	8	24.0	76.0	42.6	17.9	39.6	38.0
Fat		Control	8	46.5	165.5	96.1	38.7	89.1	92.5
		15	8	70.5	173.5	110.2	38.1	104.5	108.5
		30	8	60.0	213.5	118.6	50.2	109.6	116.0

Studies conducted in support of the COMPUDOSE implants.

COMPUDOSE implants containing either 24 mg or 45 mg of estradiol-17β provide a continuous release of the hormone of 1.25 to 2.5 μg/hour for either 200 days or 400 days at least. Excretion rates of estrogen in female cattle, for comparison, range from 7.8 μg/hour to 31.8 μg/hour in cycling heifers, depending upon the phase of the cycle, and range from 300 μg/hour during the second trimester of pregnancy to 3400 μg/hour within 15 days of parturition.

In cattle, 84% of the estradiol-17β is converted into the non-estrogenic metabolite estradiol-17α which is excreted as either the glucuronide or the sulfate conjugates by the liver (FAO, a).

Analytical Methodology

Fat tissues are extracted with hexane and chlorobutane. Liver, kidney and muscle tissues are extracted with 80/20 acetonitrile/methanol and the sulfate and glucuronide conjugates hydrolysed enzymatically. Extracts are purified by liquid-liquid partitioning and by column chromatography. Estradiol is separated from estrone by chromatography on Sephadex LH 20. The determinative step is a radioimmunoassay method using specific antisera.

The range of procedural recoveries was high and inconsistent in some studies. Extraction of all samples was monitored for individual recovery based on the recovery of an added radiolabelled standard. If the radiolabel recovery indicated high process losses, the samples were re-analysed. It is surprising that no adjustment of the results for recovery was undertaken prior to statistical analyses in this work. The transferability of the method to an independently operated second laboratory has been demonstrated (Sieck and Turner. 1981).

The limit of detection (LOD) is reported to be approximately 5 ng/kg. Below LOD results are typically reported as < 5ng/kg or an arbitrarily assigned value, of up to 5, throughout most residue studies (FAO, b).

Estrogens in kidney fat of untreated slaughter steers

The effects of location, breed, carcass weight and federal grade on distribution functions of endogenous estradiol-17β and estrone in kidney fat of slaughter steers were determined in a study with steers from seven geographical locations within the U.S.A. (2 from Alabama, 1 from each of Oklahoma, Texas, Indiana, Idaho, and Nebraska) (Frank *et al.*,

undated). Results are shown in Figure 8 and Table 27. Analytical results were corrected for recovery, determined with added isotopically labelled hormone. The levels found are possibly heterogeneous across location and/or breed and/or carcass weights. There seems to exist a linear relationship between estrone and estradiol levels, which is influenced by unknown factors (see Figure 8, where the data are labelled according to the seven regions). Information on animal characteristics was too limited to investigate possible factors.

Figure 8. **The effects of location, breed, carcass weight and federal grade on distribution functions of endogenous estradiol-17β and estrone in kidney fat of slaughter steers from seven geographical locations within the U.S.A.**

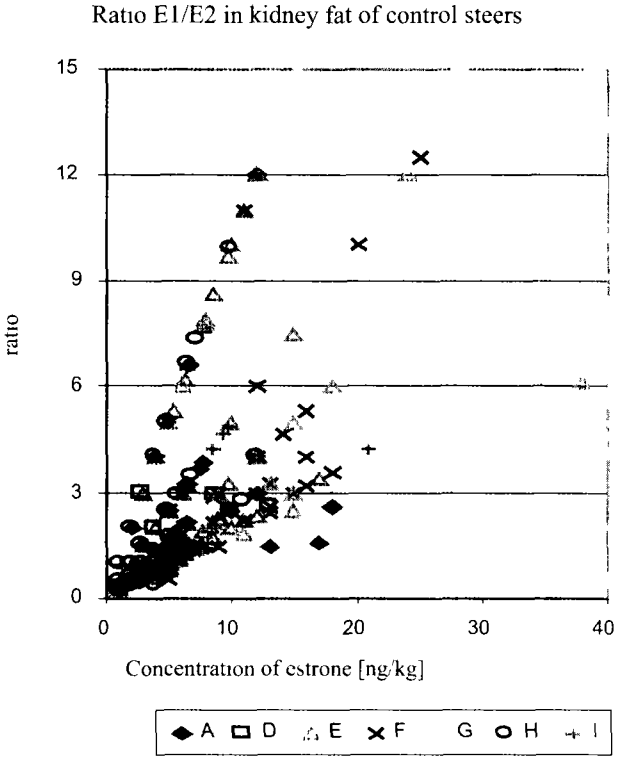


Table 27. **The effects of location, breed, carcass weight and federal grade on distribution functions of endogenous estradiol-17β and estrone in kidney fat of slaughter steers from seven geographical locations within the U.S.A.**

Statistical parameter	Concentration (ng/kg)		Ratio E_1/E_2
	Estrone	Estradiol	
n	307	306	
minimum	1.0	1.0	
maximum	38	11	
mean	6.5	3.6	1.8
standard deviation	4.78	1.71	2.8
geometric mean	5.2	3.1	1.7
median	5.0	4.0	1.3
75^{th} percentile	7.8	5.0	1.6
90^{th} percentile	12	5.0	2.5
95^{th} percentile	15	6	2.5
99^{th} percentile	25	9.0	2.8

Effect of implant withdrawal on hormone levels in steers

205 steers at three different geographic locations were implanted with COMPUDOSE®. After approximately 100 days perirenal fat samples were removed from 10 steers from two of the two locations (20 total) by biopsy prior to removal of the implant. For the remaining steers, implants were removed 24 hours prior to sampling of kidney fat. Samples were analysed for estradiol-17β and estrone. Twentyfour hours after implant removal the E_1 and $E_2\beta$ levels had returned to the levels found in untreated steers. Results are shown in Table 28 (Sieck *et al.*, undated).

Table 28. Concentration of estradiol-17β and estrone in steers from 3 different geographical locations in the U.S.A. implanted with COMPUDOSE®

Location	n	Withdrawal [hours]	Hormone concentration (ng/kg)	
			Estradiol-17β	Estrone
			Mean ± Standard Deviation	Mean ± Standard Deviation
Colorado	68	24	8.1 ± 3.9	5.3 ± 1.03
Georgia	10	0	12.9 ± 6.1	11.4 ± 4.1
	58	24	7.8 ± 3..9	5.1 ± 0.5
Idaho	10	0	12.2 ± 5.3	8.0 ± 1.8
	59	24	6.3 ± 2.3	5.0 ± 0.08

Residue Studies

Study APH 216A:

33 Angus and Hereford steers were implanted for 70-180 days with estradiol-17β Compudose implants. Several withdrawal times were observed prior to slaughter. The implants released approximately 70 µg/animal/day. Edible tissues were collected from treated animals and from 20 untreated control animals. All tissues were analysed for estradiol-17ß and estrone.

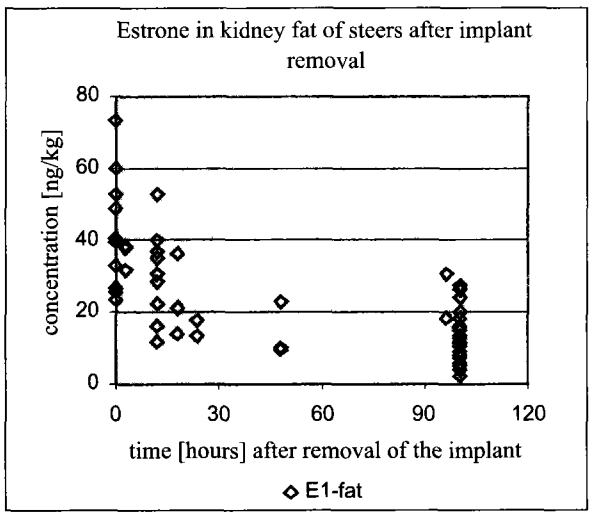
Study APH-216B

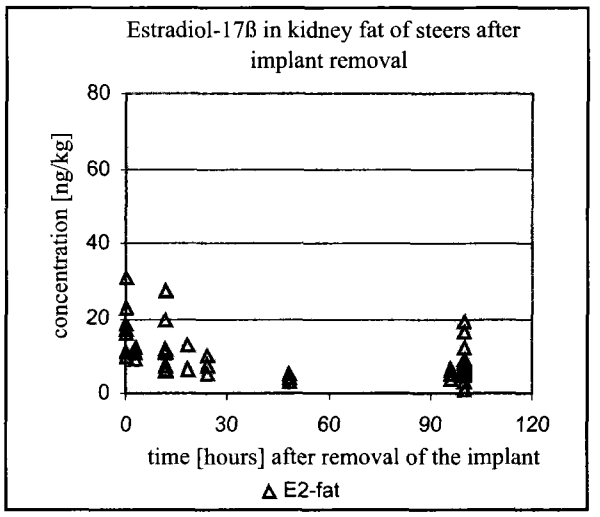
12 animals were implanted for 85 days. Implants were removed and samples were taken 0 and 12 hours after withdrawal of the implants (6 animals per group). These animals were used to provide replacement data, which was lost when the tissues of 12 animals in the first study were contaminated upon sampling.

Study APH-216E

Eight additional animals were implanted for 69-70 days to provide further information on the effects of a withdrawal time. The Figures 9a and 9b show, using fat data as an example, the rapid time-dependent decrease of hormone level following implant withdrawal (FAO, c).

Figure 9. Decrease of hormone levels in fat following the withdrawal of implant in Study APH-216E





Data for the combined studies described above is shown in Table 29. The 100-hour group appears as control in the study protocol and as 100 group in the annex with the raw data. This latter designation was originally done to facilitate line printer plots of the data. The 100-hour animals were, in fact, control animals

Table 29. Effect of implantation of steers with COMPUDOSE implants and of implant removal on hormone concentrations in edible tissues

Tissue	Kidney fat				Kidney				Liver				Muscle			
hours after implant removal	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Hormone	Estrone		Estradiol-17β		Estrone		Estradiol-17β		Estrone		Estradiol-17β		Estrone		Estradiol-17β	
N	10	24	10	24	10	29	10	24	10	27	10	26	6	20	6	20
	Hormone concentration (ng/kg)															
Minimum	23.3	2.1	9.6	1.3	10.0	1.2	12.0	1.5	4.4	1.9	1.9	1.9	3.9	1.1	2.1	2.4
Maximum	73.3	27.3	31.1	19.1	42.1	38.2	45.9	23.7	57.9	22.6	40.4	17.9	20.6	22.9	11.7	28.9
Mean [1]	39.4	12.2	19.6	7.5	24.5	10.4	28.4	10.1	18.7	9.5	17.5	6.7	10.2	8.8	7.3	11.4
S.D.	18.4	6.9	6.4	4.0	9.5	6.7	9.0	5.8	15.4	5.5	15.1	3.3	6.6	5.7	3.7	6.3
Geometric Mean	39.6	10.4	18.6	6.6	22.7	8.6	26.9	8.5	14.6	8.0	11.4	5.9	8.5	6.9	6.3	9.7
Median	39.5	10.9	20.9	6.3	24.2	9.1	29.9	9.4	15.1	8.3	9.5	5.9	9.1	8.4	7.4	10.4
Mean [2]	34.8	10.5	15.3	6.8	19.2	7.9	21.1	6.7	8.8	6.5	10	4	10.4	4.8	3.4	5.8
Factor[3]	1.1	1.2	1.3	1.1	1.3	1.3	1.3	1.5	2.1	1.5	1.8	1.7	1.0	1.8	2.1	2.0

[1] basis: corrected results [2] basis: uncorrected results [3] = $mean_{corr}$ /$mean_{uncorr}$

Study APH-216 C

In a further study, 4 steers weighing approximately 715 pounds were implanted for 90 to 147 days. Biopsy samples of kidney fat were collected at six withdrawal times after implant removal. The results are given in Table.30.

Table 30. Estrogens in kidney fat of steers implanted with COMPUDOSE as a function of the time after implant withdrawal

Withdrawal time: (hours)	0		12		24		36		72	
	Concentration (ng/kg)									
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
n	4	4	12	12	23	23	29	29	9	9
Minimum	8.9	<LOQ	5.0	1.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Maximum	50.5	8.8	21.0	6.1	16.0	5.7	17.0	7.8	20.0	25.0
Mean	23.5	6.5	10.5	<LOQ	7.8	<LOQ	5.6	<LOQ	10.6	7.1
S.D.	18.5	2.1	5.3	1.7	3.9	1.6	<LOQ	<LOQ	6.2	7.5
Geometric Mean	19.1	6.2	9.4	<LOQ	6.7	<LOQ	<LOQ	<LOQ	8.8	<LOQ
Median	17.3	6.6	9.1	<LOQ	8.4	<LOQ	<LOQ	<LOQ	9.3	<LOQ

Effects of overdosing of steers

Eight of 16 Hereford steers weighing approximately 850 lbs received four implants per animal, delivering an estimated total of 644 μg of estradiol-17β daily per animal. The remaining animals served as controls. Kidney fat tissue samples (biopsy) were taken 49-50 days after implantation. The estrone levels found in treated animals ranged from 32 to 60 ng/kg. The range of concentrations in control animals ranged from 5-6.5 ng/kg (Linsey *et al.*, undated).

Estradiol-17α in steer tissues

Tissues were collected from untreated steers, from steers having implants in place and from steers from which the implants had been removed 12 hours prior to slaughter. Tissues were analysed by RIA with an antiserum exhibiting cross-reactivities to estradiol-17α of 12, 4, and 1% by estrone, estradiol-17β and estriol, respectively. The RIA used with chromatographic fraction enriched in estradiol-17α. Residues in liver and kidney appear in Table 31 (Hendrix and Franks, undated): Concentrations in muscle and kidney fat were below the limit of detection (ca. 5 ng/kg) at all times.

Table 31. Concentration of estradiol-17α in steers with COMPUDOSE implants and in controls.

Experiment	Liver		Kidney	
	Concentration (ng/kg)			
Control	<10		<10	12.5
Zero withdrawal time	35	71.4	57.8	53.3
12 hours withdrawal time	<10	<10	15.1	<10

Residues in implanted Heifers

Estrogen concentrations in kidney fat of cycling and pregnant heifers

For the Compudose studies, kidney fat was collected at the slaughterhouse from 14 cycling and 12 pregnant heifers and assayed for estrone and estradiol-17β. Results are shown in Table 32.

Mature Hereford heifers weighing approximately 800 pounds (363.2 kg) were divided into one control group (12 animals) and one group (6 animals) which was implanted with a single COMPUDOSE 200 implant (24 mg estradiol-17β) and slaughtered 84 days after implantation. Hormone concentrations were determined in muscle, liver tissues, kidney and perirenal fat (Sieck *et al.*, undated, b). Results are summarised in Table 33.

Table 32. Estrogen concentration in kidney fat from 14 cycling and 12 pregnant heifers (ng/kg)

Reproduction status	n	Estrone			Estradiol-17β		
		Mean	Min.	Max.	Mean	Min.	Max.
Cycling	14	44	5	176	10	5	28
Pregnant							
1st trimester	3	40	11	100	5	5	6
2nd trimester	4	964	380	1920	22	5	50
3rd trimester	3	3870	750	8200	163	22	440

Table 33. Comparison of estrogen levels in mature Hereford heifers comprising one control group of 12 animals and one group of 6 animals implanted with a single COMPUDOSE 200 implant.

Tissue	Group	n	Concentration of hormones (ng/kg)	
			Estrone	Estradiol-17β
			Mean ± SD	Mean ± SD
Lean muscle	Control	11	5.9 ± 1.8	7.1 ± 3.3
	0 –hour	6	6.4 ± 2.2	5.8 ± 1.1
Liver	Control	12	7.5 ± 4.8	8.2 ± 4
	0-hour	6	6.7 ± 2.4	7.3 ± 2.5
Kidney	Control	11	6.5 ± 3	8.5 ± 4
	0-hour	6	10.2 ± 6.4	26.3 ± 11.9
Perirenal fat	Control	12	5.3 ± 1.1	5.6 ± 1.1
	0-hour	6	9.9 ± 6.7	9.3 ± 4.7

Residues in implanted bull calves

Sixty Holstein intact bull calves with an initial average weight of approximately 42.7 kg were divided into one control group and two treatment groups of 20 animals each. The implanted groups were slaughtered 56 days after implantation with no removal of the implant in one group and removal of the implant 24 hours prior to slaughter in the second treatment group. Estrogen levels were measured in kidney, lean, kidney fat, and liver of control animals and treated animals. The results of the experiment are shown in Table 34. Implant withdrawal of 24 hours were sufficient to bring the increased estrogen concentrations down to control levels (Sieck *et al.*, undated, c).

Table 34 Estrogens in tissues of bull calves implanted with COMPUDOSE

Concentration of hormone (ng/kg)	Tissue and time after implant removal											
	Muscle			Liver			Kidney			Perirenal fat		
	C	0	24	C	0	24	C	0	24	C	0	24
Estrone (n =)	17	17	16	17	16	16	17	17	16	16	17	15
Minimum	5.0	5.0	5.0	5.0	9.6	5.0	5.0	20.1	4.8	7.6	9.9	5.0
Maximum	36.5	23.3	16.6	15.0	34.6	18.4	19.1	59.9	15.4	25.0	60.8	19.8
Mean	10.8	11.7	8.0	9.1	22.3	9.2	9.8	34.7	9.6	13.9	24.6	10.4
Standard Deviation	8.71	5.72	4.11	3.56	8.85	4.38	4.55	9.77	3.66	4.88	12.07	4.85
Geometric mean	8.6	10.4	7.2	8.5	20.4	8.3	8.8	33.5	8.9	13.1	22.4	9.3
Median	7.0	11.4	5.3	9.1	22.8	8.5	8.4	34.5	9.7	13.2	23.3	10.4
Estradiol 17β (n=)	17	17	16	17	17	16	17	17	16	16	17	15
Minimum	5.0	7.6	5.0	5.0	13.6	5.0	5.0	22.8	5.6	5.0	16.5	5.0
Maximum	17.1	30.5	10.1	29.4	56.4	31.6	24.6	86.9	20.4	63.9	56.9	12.7
Mean	6.8	18.4	6.1	14.7	32.0	16.0	10.6	57.0	10.9	11.2	38.5	7.1
Standard Deviation	3.45	5.60	1.70	5.76	11.23	8.33	5.28	18.08	4.20	14.94	10.94	2.87
Geometric Mean	6.2	17.5	5.9	13.6	30.1	13.6	9.5	53.7	10.2	7.7	36.8	6.7
Median	5.0	18.8	5.3	14.0	31.3	15.7	10.5	55.8	9.9	5.3	39.9	5.2

C = control, 0 = zero hours withdrawal, 24 = 24 hours withdrawal

Residues in implanted bulls

A study was conducted to determine the tissue estrogen levels resulting from treatment of bulls with COMPUDOSE (containing 45 mg estradiol-17β per implant). Twelve Hereford bulls weighing approximately 385 kg were used in the study. Six animals were implanted; the other six served as a control. Both groups were slaughtered 63 days after

implantation. Muscle, liver, kidney and perirenal fat were analysed for estrogens and results of the study are shown in Table 35. The daily weight gain was higher than observed in steers and heifers and the corresponding increases in estrogen levels were low in muscle and liver (Decker and Turner, undated).

Table 35. Levels of estrone and estradiol-17β in Hereford bulls implanted with COMPUDOSE

Tissue:	Muscle				Liver				Kidney				Perirenal fat			
Treatment group	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I
Hormone:	Estrone		Estradiol		Estrone		Estradiol		Estrone		Estradiol		Estrone		Estradiol	
Parameter	Concentration (ng/kg)															
n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Minimum	5	5	5	5.6	5	5	5	5	5.3	8.6	5.6	9.4	5	14.7	6.2	13.3
Maximum	12.0	9.0	9.4	12.3	7.9	11.8	12.5	38.5	12.8	23.8	15.3	25.9	25.0	41.8	11.7	28.5
Mean	7.7	6.9	6.3	8.5	6.0	6.9	8.5	16.6	8.7	15.1	10.0	19.9	15.3	29.6	9.1	20.2
Standard Dev.	3.1	1.9	2.0	2.7	1.2	2.6	3.2	12.4	3.0	5.4	3.4	6.2	8.2	10.4	2.0	6.2
Geometric Mean	7.2	6.6	6.0	8.1	5.9	6.6	7.9	12.9	8.3	14.4	9.5	18.9	13.3	27.8	8.9	19.4
Median	7.2	6.8	5	8.0	5.5	6.1	8.7	15.3	8.5	13.6	9.7	20.9	13.6	33.6	9.0	19.1

C = control group, I = Implanted group

Residues in implanted Zebu steers

A tissue residue study was carried out with 12 Brazilian Zebu steers comprising 3 groups of 4 control animals, 4 animals implanted and slaughtered without prior implant removal and 4 animals slaughtered after 24 hours withdrawal of the implants. COMPUDOSE implants containing 45 mg estradiol-17β were used. The period during which the animals were implanted is not given in the report (Sieck and Turer, undated). Results are summarised in Table 36.

Table 36. Estrogen levels in Zebu steers implanted with COMPUDOSE

Tissue:	Perirenal fat						Liver						Muscle					
Treatment:	C	0	24	C	0	24	C	0	24	C	0	24	C	0	24	C	0	24
Hormone:	Estrone			Estradiol-17β			Estrone			Estradiol-17β			Estrone			Estradiol-17β		
Parameter	Concentration (ng/kg)																	
Minimum	<5	<5	<5	<5	5.9	<5	6.3	7.4	<5	<5	9.5	6.8	13.8	<5	<5	8.4	<5	<5
Maximum	6.5	11.4	<5	<5	20.0	<5	13.9	16.6	17.3	34.5	20.4	15.5	28.8	16.3	20.0	27.5	12.0	6.4
Median	5.3	7.65	<5	<5	6.65	<5	8.0	10.6	10.4	8.1	13.9	10.3	16.8	9.75	9.45	8.6	5.45	5.65

C = control, 0 = 0 h withdrawal, 24 = 24 h withdrawal

Effects of Trenbolone-containing Implants on Estrogen Levels in Tissues of Treated Animals

Estradiol benzoate/progesterone implants and trenbolone implants are approved for use in steers for improved feed efficiency. The concomitant use of estradiol implants and of trenbolone implants (Implix/Revalor) was investigated in one study, however, it does not represent an authorised usage. All the remaining studies reported here have been conducted with products containing the substances in fixed combinations.

Studies using Finaplix® implants in heifers

Methodological aspects

Free steroids were extracted from crude tissue extracts with toluene:ether (7:3); conjugated steroids were extracted by the same mixture following hydrolysis with glucuronidase/sulfatase (*helix pomatia*). A further cleanup-step was performed on reverse phase C-18 cartridges. The steroids were then separated by HPLC and quantified by RIA. The analytical detection limits for free estradiol-17β, conjugated estradiol-17β and free estrone in tissues were 18%, 25% and 27%, respectively. Recoveries of free estradiol-17β and conjugated estradiol-17β were 30-40% and 25-30%, respectively. Recovery of the extraction was estimated using ^{3}H-estradiol-17β (free hormone) and ^{3}H-testosterone glucuronide (conjugated hormone) and results of recovery experiments are summarised in Table 37.

During the study, it was observed that, at certain times, the heifers were in estrus. Where deviations in estradiol levels were found, when compared with the mean estradiol levels of the group, the phase of the menstrual cycle may be one of the reasons

Table 37. The recoveries of estradiol-17β and estrone using Finaplix® implants in heifers

Analyte	muscle (n=9)		liver (n=9)		Kidney (n=9)	
	level (ng/kg)	CV (%)	level (ng/kg)	CV (%)	Level (ng/kg)	CV (%)
Free estradiol-17β	23	20	21	24	48	14
	13	36	5	68	6	99
Conjugated estradiol-17β			127	9	46	17
			21	40	15	43
Free estrone			15	52		
			107	12		

Residue study in heifers

Twenty-four heifers (average age 1.5 years; 270-295 kg BW) were implanted with Finaplix® (300 mg trenbolone acetate). Groups of 6 animals were slaughtered at 15, 30, 60, and 75 days after implantation, respectively (Arts *et al.*, 1986a). Unfortunately, there was no control group in the study. Only the results of the determination of estrogen concentrations are considered in this review. The concentrations of free and conjugated estradiol-17β in muscle, liver, kidney, fat, and blood plasma were determined. Free estrone was only determined in liver and fat. The method was not sensitive enough to reliably measure the endogenous hormone levels.

No clear trend can be identified from the results of the study because of the insensitivity of the method, the high variability of the results, the small group sizes and absence of untreated controls. There is possibly a trend in direction of decreasing estrogen levels in liver and fat. Table 38 shows the individual estrogens and the median of the sum of all three compounds measured (n=6).

Table 38. Effects of implantation with FINAPLIX® on the median estrogen levels in tissues of heifers

Days Withdrawal	Tissue:	Muscle	Liver	Kidney	Fat
	Analyte	Concentration (ng/kg)			
15	E_2, free	15.5*	17.0*	12.5*	47.5
	E_2, conjugated	11.0*	88.5	26.5	14.0*
	E_1, free		29.5		33
	Sum of estrogens	36.0	137.5	38.5	89.0
30	E_2, free	22.5	27.0	11.5*	40.5
	E_2, conjugated	22.0*	88.0	15.5*	14.5*
	E_1, free		24.5*		54.5
	Sum of estrogens	44.5	129.0	31.5	112.0
60	E_2, free	7.0*	22.5	20.5	25.5
	E_2, conjugated	3.0*	40.0	17.5*	13.0*
	E_1, free		22		34.5
	Sum of estrogens	9.5	86.5	38.0	73.5
75	E_2, free	9.5*	18.5	30.5	8.0*
	E_2, conjugated	11.5*	23.5*	40.5	0.0*
	E_1, free		18*		38
	Sum of estrogens	28.5	62.5	75.5	45.0

* = values are below the LOQ for the given analyte, E_2 = estradiol-17β

Studies using Torelor® implants

Twenty four steers (average age 2.5 years; 354-459 kg BW) were treated with Torelor® (200 mg trenbolone acetate, 40 mg estradiol-17ß); 6 other animals served as a control group. Groups of six treated animals were slaughtered at 15, 30, 60, and 75 days, respectively, following the time of implantation. Two control animals were slaughtered at 15, 30, and 60 days following implantation of the treated animals. Tissue and blood samples were taken and analysed using the method already described above. Blood plasma levels were monitored over all time frames, with groups slaughtered on the same day after implantation (Arts *et al.*,1986b). The results are shown in Table 39.

In a further study with TORELOR®, 24 steers (approximately 430 kg; 2 years old) were implanted twice with an intermediate period of 60 days (Arts *et al.*, 1986c). One group of six steers was slaughtered 60 days after the first implantation. Three other groups of six animals each were re-implanted on day 60 and slaughtered on days 15, 30, and 60, respectively, after the second implantation. The results of this work are shown in Table 40.

Studies in veal calves using Implix BM, Implix BF and Revalor lactose

Studies in veal calves using Implix BM, Implix BF and Revalor lactose have been reported (Roberts and Cameron, 1986). The report contains no data on method validation. Friesian calves (39 male and 39 female), three weeks old at implant, were divided into three groups for treatment. Group 1 animals, comprised of 21 males and 21 females, were dosed with Implix BM (males) and Implix BF (females), respectively, on day 0 of the study. On day 50 the animals were dosed with Revalor lactose. Group 2, comprised of 12 animals of each sex, were dosed with Revalor lactose on day 50. Group 3, comprised of 6 animals of each sex, served as untreated controls. Calves were killed at various intervals. Results are summarised in Table 41

Table 39 **Estrogen concentrations (ng/kg) in steers implanted with TORELOR®**

Treatment group	Tissue	N	Min	Max	Mean	S.D.	Geomean	Median	
Control	Muscle	6	13.0	46.0	22.5	12.4	20.3	17.5	free E_2
		6	0.0	25.0	9.3	8.4	0.7	8.0	conjugated E_2
		6	15.0	51.0	31.8	13.2	29.4	31.5	total E_2
	Liver	6	14.0	31.0	20.5	6.9	19.6	17.5	free E_2
		6	24.0	51.0	34.5	9.2	33.6	33.5	conjugated E_2
		6	43.0	66.0	55.0	9.2	54.4	54.0	total E_2
		5	12.0	22.0	16.4	4.0	16.0	17.0	estrone
	Kidney	6	12.0	20.0	15.5	3.4	15.2	14.5	free E_2
		6	35.0	47.0	41.0	4.1	40.8	41.5	conjugated E_2
		6	47.0	67.0	56.5	7.1	56.1	56.5	total E_2
	Fat	6	2.0	22.0	13.7	7.4	10.8	15.5	free E_2
		6	0.0	17.0	9.7	7.9	0.1	12.5	conjugated E_2
		6	2.0	39.0	23.3	14.7	16.1	29.5	total E_2
		6	7.0	31.0	24.0	9.6	21.5	28.5	estrone
15 days implant	Muscle	6	12.0	100.0	40.2	31.1	32.4	34.5	free E_2
		6	1.0	38.0	14.3	13.7	8.4	10.5	conjugated E_2
		6	22.0	138.0	54.5	43.0	44.6	40.0	total E_2
	Liver	6	8.0	68.0	40.2	24.8	30.5	50.0	free E_2
		6	39.0	710.0	296.3	252.6	186.7	276.5	conjugated E_2
		6	50.0	756.0	336.5	268.6	220.0	337.5	total E_2
		6	11.0	116.0	53.2	37.1	41.2	53.5	estrone
	Kidney	6	35.0	66.0	53.2	10.3	52.2	53.5	free E_2
		6	67.0	146.0	98.7	31.0	94.8	91.0	conjugated E_2
		6	121.0	212.0	151.8	37.1	148.3	140.0	total E_2
	Fat	6	94.0	141.0	119.2	17.2	118.1	120.0	free E_2
		6	0.0	18.0	13.7	6.8	1.0	15.5	conjugated E_2
		6	112.0	157.0	132.8	18.4	131.8	133.5	total E_2
		6							estrone
30 days implant	Muscle	6	15.0	82.0	38.7	24.4	32.9	36.0	free E_2
		6	2.0	44.0	20.0	15.8	12.7	20.0	conjugated E_2
		6	33.0	126.0	58.7	34.4	52.6	47.0	total E_2
	Liver	6	5.0	148.0	61.5	48.2	41.9	60.5	free E_2
		6	37.0	525.0	306.0	191.7	226.6	339.0	conjugated E_2
		6	42.0	586.0	367.5	195.5	284.6	384.5	total E_2
		6	13.0	81.0	59.5	24.6	51.7	62.5	estrone
	Kidney	6	42.0	68.0	53.0	10.2	52.2	53.0	free E_2
		6	77.0	171.0	117.2	36.2	112.5	115.5	conjugated E_2
		6	119.0	239.0	170.2	42.6	165.8	172.0	total E_2
	Fat	6	68.0	176.0	122.0	40.9	116.1	115.0	free E_2
		6	0.0	22.0	12.7	10.3	0.7	15.5	conjugated E_2
		6	90.0	196.0	134.7	44.4	129.0	115.5	total E_2
		6	76.0	110.0	92.5	12.2	91.8	91.0	estrone

Table 39 continued. Estrogen concentrations (ng/kg) in steers implanted with TORELOR®

Treatment group	Tissue	N	Min	Max	Mean	S.D.	Geomean	Median	
60 days implant	Muscle	6	16.0	56.0	37.2	14.7	34.4	38.0	free E_2
		6	0.0	13.0	8.2	4.5	0.7	9.0	conjugated E_2
		6	27.0	65.0	45.3	15.2	43.1	47.0	total E_2
	Liver	6	13.0	41.0	25.2	9.3	23.7	23.5	free E_2
		6	77.0	955.0	295.3	333.1	198.9	167.0	conjugated E_2
		6	101.0	996.0	320.5	340.6	228.0	188.0	total E_2
		6	20.0	139.0	57.0	42.4	47.1	49.5	estrone
	Kidney	6	20.0	94.0	50.8	28.6	44.4	41.5	free E_2
		6	11.0	380.0	111.3	140.2	55.1	66.5	conjugated E_2
		6	31.0	474.0	162.2	166.2	106.6	105.5	total E_2
	Fat	6	74.0	180.0	125.5	46.5	118.2	118.0	free E_2
		6	2.0	57.0	23.7	18.2	16.5	21.0	conjugated E_2
		6	97.0	201.0	149.2	45.5	143.1	150.5	total E_2
		6	59.0	106.0	84.5	16.2	83.1	88.0	estrone
75 days implant	Muscle	6	8.0	47.0	27.3	16.5	22.5	27.0	free E_2
		6	6.0	16.0	11.0	3.5	10.5	11.0	conjugated E_2
		6	17.0	59.0	38.3	17.8	34.7	35.0	total E_2
	Liver	6	13.0	27.0	20.3	4.6	19.9	20.5	free E_2
		6	26.0	375.0	98.5	136.8	57.9	36.5	conjugated E_2
		6	48.0	402.0	118.8	140.3	81.8	55.5	total E_2
		6	13.0	132.0	36.2	47.1	23.6	18.0	estrone
	Kidney	6	21.0	126.0	73.8	43.1	61.6	71.5	free E_2
		6	32.0	383.0	133.3	129.1	96.2	90.0	conjugated E_2
		6	53.0	497.0	207.2	162.7	160.1	161.5	total E_2
	Fat	6	32.0	175.0	83.7	60.0	67.1	61.5	free E_2
		6	0.0	18.0	8.5	9.3	0.0	8.0	conjugated E_2
		6	32.0	191.0	92.2	65.8	74.4	65.0	total E_2
		6	38.0	109.0	68.5	26.2	64.2	74.0	estrone

. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

Table 40. Estrogens in plasma and tissues of steers implanted with TORELOR®

Time	Tissue	Muscle			Liver				Kidney			Fat				Plasma		
	Substance	Free E_2	Conjug. E_2	Total E_2	Free E_2	Conjug. E_2	Total E_2	E_1	Free E_2	Conjug. E_2	Total E_2	Free E_2	Conjug. E_2	Total E_2	E_1	Free E_2	Conjug. E_2	Total E_2
		Concentration (ng/kg)																
Day 60	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		6
	Minimum	14	0	15	10	22	32	5	49	90	139	73	1	77	39	4		4
	Maximum	45	3	48	81	651	732	94	84	244	307	177	10	180	69	18		18
	Mean	30	1	31	41.8	279	321	35.6	65.6	175	240	124	4.4	129	54.8	11		11
	Stand. Dev.	11.9	1.22	12.5	26.9	240	267	34.4	12.5	61.9	69.8	38.5	3.36	37.3	11.2	5.70		5.70
	Geom.Mean	27.9		28.8	33.7	174	211	23.9	64.6	165	231	119	3.44	124	53.8	9.64		9.64
	Median	30	1	31	44	257	301	22	65	197	262	128	4	132	53	11		11
Day 75	n	5	5	6	5	5	6	5	5	5	6	5	5	6	5	5		6
	Minimum	25	0	0	3	52	0	11	46	60	0	105	0	0	35	6		0
	Maximum	36	2	38	51	491	542	56	117	295	412	184	10	184	97	16		16
	Mean	30.3	0.5	24.6	24	205	183	25.5	73.3	164	190	130	2.75	106	65.8	9.25		7.4
	Stand. Dev.	4.79	1	14.6	20.7	195	213	20.9	30.6	97.7	154	37.1	4.86	67.5	25.7	4.57		5.73
	Geom.Mean	30.0			15.6	149		20.5	69.0	141		127			61.7	8.56		
	Median	30	0	28	21	139	139	17.5	65	151	200	116	0.5	106	65.5	7.5		7
Day 90	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	3	6
	Minimum	73	5	110	9	101	122	14	73	131	204	164	2	169	46	10	4	10
	Maximum	135	43	177	98	1074	1172	194	137	303	422	401	15	416	119	126	9	135
	Mean	100	29.4	130	37.6	328	366	57	109	239	348	284	8.2	292	88.6	46.2	5.67	49.6
	Stand. Dev.	24	16.5	27.6	37.1	418	452	77.0	30.7	72.0	88.1	88.7	4.97	92.1	30.0	49.3	2.89	52.7
	Geom.Mean	98.1	23.2	128	25.4	209	240	33.1	105	229	337	272	6.7	280	83.9	28.9	5.24	30.8
	Median	105	37	126	21	161	177	27	127	277	381	290	9	292	92	20	4	24
Day 120	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6
	Minimum	48	19	94	10	47	63	14	56	110	166	111	1	112	65	9	11	19
	Maximum	128	50	178	25	193	207	57	106	211	303	293	8	298	109	19	19	29
	Mean	86.6	41.6	128	17	102	119	28.4	83.2	155	239	194	4	198	80.6	13.2	14.5	24.8
	Stand. Dev.	29.8	12.7	34.3	5.74	59.6	60.0	17.9	19.9	39.1	53.8	75.3	2.74	77.5	19.2	4.38	3.70	4.49
	Geom.Mean	82.3	39.4	125	16.2	89.4	108	24.6	81.1	152	234	182	3.17	186	78.9	12.6	14.2	24.5
	Median	83	46	121	16	85	110	18	92	153	223	160	4	164	70	13	14	27

Table 41. **Concentration (ng/kg) of estradiol-17β, estradiol-17α, progesterone and testosterone in tissues of control and calves implanted with Implix BM, Implix BF and Revalor lactose**

Analyte	Day	Muscle			Liver*			Liver			Kidney			Fat		
		Control	Revalor only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor
Estradiol in males	15			36.1			954			154			85			91.9
	30	7.3		48.3	574		682	32.1		75.6	13		125	11.8		38.3
	50			60.3			824			193			134			95.6
	65		94.4	114		1105	1300	53.3	139	179		154	208		96.4	159
	80	20.2	117	106	685	1323	1284		160	206	9.2	177	268	23.1	60	122
	100		112	172		666	2334		106	226		172	250		50.9	206
Progesterone in males	15			606						599			994			5407
	30	901		597				749		924	4066		2798	1598		6520
	50			772						771			1409			8664
	65			268						20245			1582			15534
	80	409		482				1855		633	1022		1221	2662		6933
	100			500						442			1460			7996
		Control	Revalor only	Implix BM, Revalor	Control	Revalor Only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor	Control	Revalor only	Implix BM, Revalor
Estradiol in females	15			106.5			1790			512			249			75.2
	30	5.6		88.5	601		703	32.4		72.6	17		170	15.2		171
	50			88.6			698			95.4			146			119
	65		84.9	156.4		875	1721		79.6	271		144	163		16.1	139
	80	14.6	100.7	155.4	589	1114	1560	23.4	148	149	18.5	198	451	18.8	129	132
Testosterone in females	15			360						196			588			1027
	30	6.1		246				108		66.4	95.5		564	22.2		1259
	50			226						71.2			515			750
	65			188						79.4			708			587
	80	8.5		179				101		72.2	238		637	77.2		722

- = estradiol-17 α
-

Studies using REVALOR implants

Therre are several types of Revalor® implants available. These are listed below

- Revalor® -G for feedlot steers: The recommended dosage, one implant, contains 40 mg trenbolone acetate and 8 mg estradiol in two pellets
- Revalor® -S for feedlot steers; one implant contains 120 mg trenbolone acetate and 24 mg estradiol in six pellets.
- Revalor-H® for heifers: One implant contains 7 pellets (140 mg trenbolone acetate and 14 mg estradiol-17β) for use in heifers fed in confinement (feedlot).

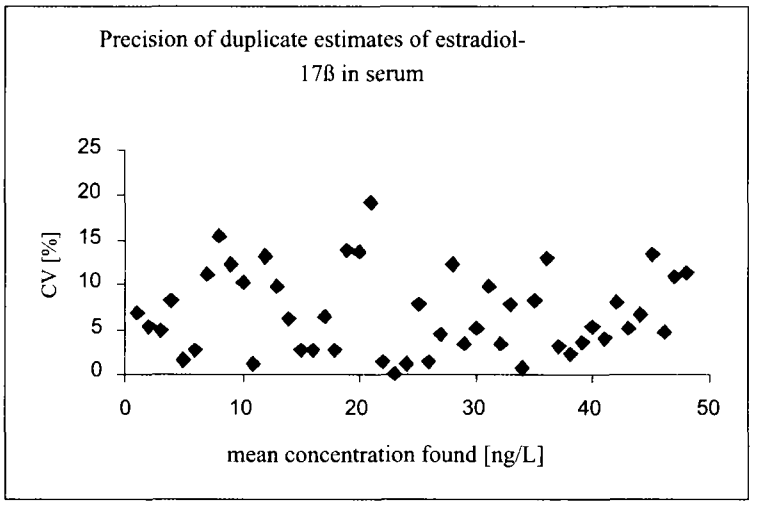
In a study by Wray (1986), twelve steer calves of primarily Angus, Herford and various continental breeds (initial average weight 563 pounds on day five of the study, and 788 on the last day) were used as controls (4 animals) or treated by implantation (8 animals). Blood samples were taken twice from each animal on days -1, 0, 14, 28, 42, 56, 70, 84, 98, 112 and serum was obtained for analyses. The analyses were performed by Clemson University. The report concluded: "While hardly any significant differences were found between the treatments when looking at each collection date individually, there was a significant difference between the control and treated animals over all time in both E_2-ß and T".

Method characteristics

Serum extracts were cleaned by C_{18} reverse phase columns prior to RIA. All samples were corrected for recovery. Accuracy was determined as the percent recovery using fortified tissue and averaged 103.5. However, efficiency of extraction of ^3H-E_2 (mean recovery from a subset of serum samples in each of the assays) was 87%. Inter-assay variation was 13% and intra-assay variation using replicates of all experimental samples > LOQ was 7%. No information was provided to indicate that conjugates are included in the residue determinations.

Each serum sample was assayed in duplicate using. The coefficient of variation, expressed as a percentage, of duplicate estimates calculated from these results was 6.2 for the median and 13.8 for the 95th percentile, respectively (n=47). Results are plotted in Figure 10.

Figure 10. Precision of duplicate estimates of estradiol-17β in serum analyses following REVALOR® implantation



Residues in plasma and tissues

Figure 11 shows the plasma levels determined at various times after Revalor® implantation. The results obtained with some of the individual animals suggest that the concentrations in serum reach a maximum after approximately 50 days. This conclusion is also supported if the median (or the mean, or the geometric mean) of the results obtained from all

animals is calculated and the results are plotted instead of the individual data. Table 42 shows data on the estradiol concentrations in the tissues of animals from this study.

Figure 11. Estradiol concentrations in the serum of steers implanted with REVALOR

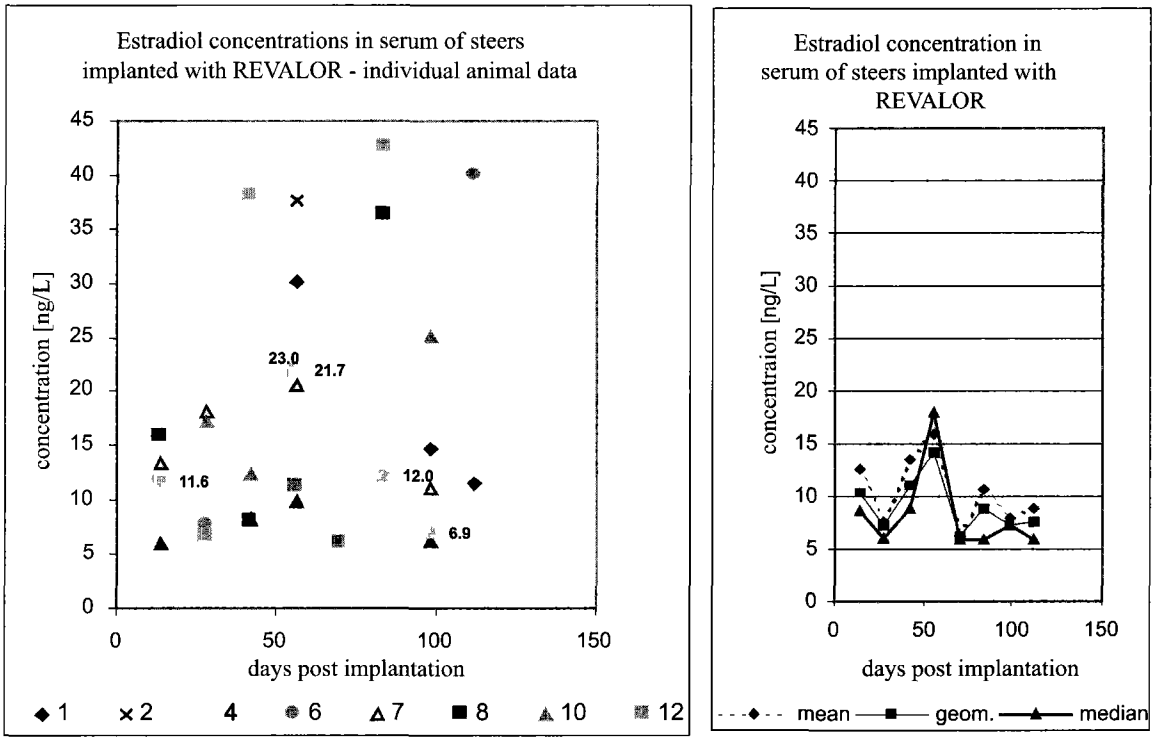


Table 42 Estradiol in tissues of steers implanted with estradiol-17ß/trenbolone acetate (REVALOR)

Withdrawal time	Concentration (ng/kg)			
	Muscle	Fat	Liver	Kidney
Control	2.1	6.6	0.0	21.2
Day 15	4.2	17.9	0.9	22.5
Day 30	4.6	14.2	0.5	19.4

A further study on tissue concentrations of estradiol-17β, trenbolone-17β, and trenbolone-17α in heifers implanted with a combination of trenbolone acetate (180 mg) and estradiol-17β (18 mg) was conducted (Clemson University. undated). Certain methodological aspects of the study remain unclear. The author states, for example: "Standard curves for the E_2-B for muscle, fat, and liver tissues were constructed with tissue extracts from control heifers. The concentration of estradiol reported in these tissues is above the background level from control heifers. Standard curves for the kidney tissue were constructed using extraction solvent residues rather than tissue residues. Concentrations of estradiol reported in kidney tissues are total levels." Results appear in Table 43.

Table 43. Estradiol in tissues of heifers implanted with estradiol-17ß/trenbolone acetate

Sample	Muscle	Fat	Liver	Kidney
	Concentration (ng/kg)			
control	0.5	0.1	0.0	17 0
day 15	3.1	23.9	4.1	35 9
day 30	7.0	26.8	11 1	40.4

CALCULATION OF THEORETICAL DAILY INTAKES

By multiplying the hormone concentrations [units/gram] found in the edible tissues with the arbitrary consumption figures established by JECFA, the theoretical daily intakes are obtained. The Theoretical Maximum Daily Intake (TMDI) is the sum of the individual daily intakes:

$$300\ c_{muscle} + 100\ c_{liver} + 50\ c_{kidney} + 50\ c_{fat} = \text{TMDI/500 g "meat"}$$

In this report the median concentration found is used for these calculations The reasons are the following. It is obvious from the raw data that the concentrations of the hormones in the tissues are not normally distributed. A logarithmic transformation of the data is not always an appropriate solution to the problems, in particular, if analytical "zero's" are included in the results and/or if the number of results below the limit of quantification is significant. For the great majority of all these cases it is still possible to calculate a median even under these conditions. Table 44 gives an example of the calculations. The data used are the results of the study with Synovex S in steers.

Table 44. Theoretical daily intake of estrone, estradiol and progesterone using median hormone levels found in and steers implanted with SYNOVEX-S and on JECFA tissue consumption figures.

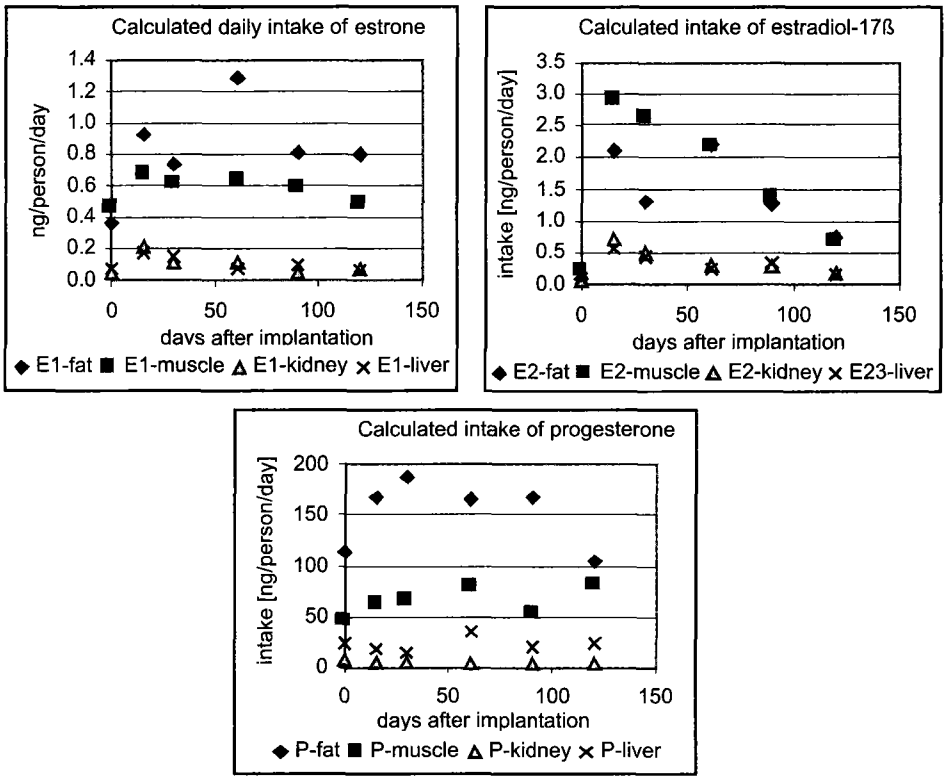
Tissue		n	Concentration (ng/kg)			Intake/day (ng)			Contribution to TMDI (%)		
			E_1	E_2	P	E_1	E_2	P			
Fat	Control	21	7.37	1.91	2290	0.37	0.10	115	39	21	60
	15	8	18.6	42.2	3345	0.93	2.11	167	47	34	66
	30	8	14.7	26.0	3725	0.74	1.30	186	46	27	68
	61	8	25.7	43.9	3290	1.28	2.19	165	61	45	59
	90	7	16.3	25.6	3350	0.82	1.28	168	52	39	68
	120	7	16.1	14.8	2110	0.81	0.74	106	56	42	49
Muscle	Control	16	1.53	0.68	150	0.46	0.20	45	48	44	24
	15	8	2.24	9.71	210	0.67	2.91	63	34	46	25
	30	8	2.04	8.74	220	0.61	2.62	66	38	54	24
	61	8	2.13	7.23	260	0.64	2.17	78	30	44	28
	90	7	1.96	4.51	180	0.59	1.35	54	38	42	22
	120	7	1.63	2.23	270	0.49	0.67	81	34	38	38
Kidney	Control	18	1.02	1.53	135	0.05	0.08	6.8	5.4	17	3.5
	15	8	4.28	14	120	0.21	0.70	6.0	11	11	2.4
	30	8	2.18	9.68	120	0.11	0.48	6.0	6.8	10	2.2
	61	8	2.21	5.96	80	0.11	0.30	4.0	5.2	6.1	1.4
	90	7	1.08	5.38	90	0.05	0.27	4.5	3.5	8.3	1.8
	120	7	1.56	3.91	90	0.08	0.20	4.5	5.4	11	2.1
Liver	Control	18	0.73	0.87	240	0.07	0.09	24	7.7	19	13
	15	8	1.73	5.51	175	0.17	0.55	18	8.7	8.8	6.9
	30	8	1.48	4.24	145	0.15	0.42	15	9.2	8.8	5.3
	61	8	0.79	2.42	340	0.08	0.24	34	3.7	4.9	12
	90	7	1.06	3.37	195	0.11	0.34	20	6.8	10	7.9
	120	7	0.64	1.4	245	0.06	0.14	25	4.5	8.0	11

Table 45 summarises the Theoretical Maximum Daily Intake resulting from the consumption of 300g muscle, 100g liver and 50g of each, kidney and fat. The TMDI would be highest if the animals would be slaughtered 15 days after implantation (see also Figures 12 a-c where the controls are plotted on day 0).

Table 45. Theoretical Maximum Daily Intake of estrone, estradiol, and progesterone resulting from the consumption of edible tissues of steers implanted with SYNOVEX-S

Days after implantation	TMDI [ng/day]		
	Estrone	Estradiol	Progesterone
Control	1.0	0.5	190
15	2.0	6.3	254
30	1.6	4.8	273
61	2.1	4.9	281
90	1.6	3.2	246
120	1.4	1.7	216

Figure 12. Theoretical Maximum Daily Intake of estrone, estradiol, and progesterone resulting from the consumption of edible tissues of steers implanted with SYNOVEX-S



Calculations of theoretical daily intake of estrone, estradiol and testosterone on the basis of median hormone levels found in control heifers and in heifers implanted with SYNOVEX-H and on JECFA consumption figures are presented in Table 46.

Table 47_summarises the Theoretical Maximum Daily Intake resulting from the consumption of 300g muscle, 100g liver and 50g of each, kidney and fat. The TMDI would be highest if the animals would be slaughtered 15 days after implantation.

Theoretical daily intakes were calculated in the same way on the data base available and described in previous paragraphs. Table 48 summarises the results obtained when the data from the pregnant heifer study were used. Depending on the duration of pregnancy at the time of implantation, treatment results in either a slight increase (estrone, late pregnancy; testosterone, early pregnancy;) or of a significant decrease (estradiol, late pregnancy) of the TMDI.

Table 49 summarises the theoretical daily intakes resulting from the consumption of edible tissues of pregnant heifers, both untreated animals and animals implanted with Synovex-H at various times of pregnancy. Table 50 summarises the theoretical daily intakes from the consumption of edible tissues obtained from calves implanted with SYNOVEX-implants.

Table 46. Calculation of theoretical daily intake of estrone, estradiol and testosterone using median hormone levels in heifers implanted with SYNOVEX-H using JECFA consumption figures.

Tissue	Day after implantation	n	Concentration (ng/kg)			Intake/day (ng)			Contribution to TMDI (%)		
			E_1	T	P	E_1	T	P			
Fat	Control	35	10	9.2	24	0.5	0.5	1.2	37	30	6.8
	30	20	36	73	290	1.8	3.7	15	47	25	21
	60	25	26	48	129	1.3	2.4	6.5	48	40	22
	89	10	29	54	132	1.5	2.7	6.6	45	42	18
	119	10	12	11	31	0.6	0.6	1.5	37	39	5.6
Muscle	Control	15	2.3	2.9	22	0.7	0.9	6.5	48	57	37
	30	20	5.3	30	101	1.6	9.1	30	41	63	44
	60	10	3.8	10	41	1.1	3.1	12	41	50	42
	89	10	4.8	10	52	1.4	3.1	16	44	48	42
	119	10	2.6	2.1	27	0.8	0.6	8.1	49	42	29
Kidney	Control	15	1.4	2.0	169	0.1	0.1	8.5	5.0	6.4	48
	30	10	5.4	23	431	0.3	1.1	22	6.9	7.8	31
	60	15	3.0	7.5	180	0.2	0.4	9.0	5.6	6.2	31
	89	10	4.0	7.7	256	0.2	0.4	13	6.1	6.0	35
	119	10	1.7	2.9	330	0.1	0.1	16	5.4	9.7	60
Liver	Control	10	1.4	1.0	13	0.1	0.1	1.3	9.8	6.5	7.6
	30	10	2.0	6.8	32	0.2	0.7	3.2	5.3	4.6	4.6
	60	10	1.4	2.2	16	0.1	0.2	1.6	5.3	3.7	5.6
	89	10	1.5	2.7	20	0.2	0.3	2.0	4.8	4.1	5.5
	119	10	1.3	1.4	15	0.1	0.1	1.5	8.6	9.3	5.4

Table 47. Theoretical Maximum Daily Intake of estrone, estradiol, and testosterone from the consumption of edible tissues of heifers implanted with SYNOVEX-H

Days after implantation	TMDI (ng/person/day)		
	Estrone	Estradiol	Testosterone
Control	1.4	1.5	17
15	3.9	15	70
30	2.7	6.1	29
61	3.2	6.5	37
90	1.6	1.5	28

Table 48. **Theoretical Maximum Daily Intake of estrone, estradiol, and testosterone from the consumption of edible tissues of pregnant heifers implanted with SYNOVEX-H**

Description of the treatment group	Duration of pregnancy at day of implantation	Days after implantation	TMDI (ng/person/day)		
			Estrone	Estradiol-17β	Testosterone
Unsynchronised controls	120		93	16	203
Synchronised controls	120		113	16	172
Implanted	120	61	34	15	233
Synchronised controls	180		280	48	282
Implanted	180	61	107	24	237
Synchronised controls	240		326	139	377
Implanted	240	61	377	49	326

Table 49. **Theoretical daily intakes from the edible tissues obtained from calves implanted with SYNOVEX-implants.**

Treatment	Hormone	A: Studies with female calves/heifers									
		Muscle		Fat		Liver		Kidney		All tissues	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
		Theoretical Intake (ng/person/day)								TMDI (ng/person/day)	
A*	E_1	0.6	0.7	1.0	0.3	0.11	0.06	0.16	0.04	1.9	1.1
	E_2	3.7	2.4	2.0	0.8	0.36	0.15	0.35	0.06	6.3	3.5
	P	134	0.7	337	0.1	9	7.27	24	14	504	22
B*	E_1	1.2	0.5	1.4	0.4	0.20	0.16	0.16	0.08	3.0	1.2
	E_2	1.7	1.4	1.4	0.5	0.36	0.18	0.29	0.10	3.7	2.2
	P	136	0.2	338	0.0	27	19	19	34	520	53
C**	E_1	1.0	0.4	1.0	0.1	0.16	0.12	0.20	0.14	2.4	0.8
	E_2	2.4	1.0	1.4	0.3	0.36	0.31	0.47	0.13	4.5	1.7
	T	z	0.6	5.9	0.2	3.2	1.9	11	11	30	13
D**	E_1	1.3	0.9	1.5	0.3	0.29	0.18	0.29	0.12	3.4	1.4
	E_2	6.9	2.5	3.8	0.8	1.3	0.75	0.94	0.34	13	4.4
	T	35	0.9	14	0.3	2.9	1.8	24	20	76	22
E**	E_1	0.6	0.4	0.8	0.1	0.12	0.10	0.13	0.09	1.7	0.7
	E_2	2.8	1.4	1.6	0.4	0.26	0.25	0.37	0.09	5.0	2.1
	T	16	0.7	9.7	0.1	2.4	1.8	14	20	42	22
F**	E_1	1	1.0	1.4	0.7	0.2	0.2	0.14	0.04	2	2
	E_2	1	2.4	2.2	0.2	0.5	0.2	0.42	0.10	4	3
	T	14	18	9.7	2.3	4.6	3.6	20	28	48	51

Table 49 (continued). Theoretical daily intakes from the edible tissues obtained from calves implanted with SYNOVEX-implants.

	A: Studies with castrated male calves/steers										
A*	E_1	0.6	0.2	0.8	0.3	0.2	0.2	0.2	0.0	1.9	0.8
	E_2	3.5	0.3	1.8	0.2	0.3	0.1	0.3	0.1	5.9	0.7
	P	259	95	310	389	6.8	6.0	35.0	10.9	611	501
B*	E_1	0.6	0.1	0.8	0.1	0.1	0.1	0.1	0.0	1.6	0.4
	E_2	1.8	0.1	1.5	0.2	0.2	0.2	0.2	0.0	3.7	0.5
	P	72	91	392	436	14	11	12	14	491	552
C**	E_1	0.8	0.6	0.6	0.2	0.2	0.2	0.2	0.1	1.8	1.1
	E_2	1.7	0.7	1.1	0.1	0.4	0.3	0.3	0.1	3.4	1.2
	P	111	152	595	489	16	10	17	18	739	669
D**	E_1	1.4	0.2	1.9	0.1	0.1	0.1	0.2	0.0	3.7	0.5
	E_2	6.5	0.4	2.8	0.1	0.4	0.3	0.9	0.1	11	0.9
	P	106	131	399	258	25	15	10	18	540	421
E**	E_1	0.8	0.7	0.9	0.2	0.2	0.1	0.2	0.1	2.1	1.2
	E_2	2.7	0.3	1.1	0.1	0.4	0.2	0.4	0.1	4.7	0.7
	P	99	163	285	345	21	13	10	14	414	536
C**	E_1	0.9	0.3	0.8	0.2	0.2	0.2	0.2	0.1	2.2	0.8
	E_2	1.7	0.3	0.9	0.2	0.4	0.4	0.4	0.1	3.4	1.0
	P	193	753	293	332	20	13	21	73	527	1170

A = implanted day 0, slaughtered day 61. **B** = implanted day 0, slaughtered day 119. **C** = implanted day 0, implanted day 118, slaughtered day 241. **D** = implanted day 0, implanted day 118, implanted day 240, slaughtered day 301. **E** = implanted day 0, implanted day 118, implanted day 240, slaughtered day 329. **F**= implanted day 0, implanted day 118, implanted day 240, slaughtered day 360.

* = testosterone not determined ** = progesterone not determined

Table 50. Theoretical daily intakes from the tissues of pregnant heifers (untreated animals and animals implanted with Synovex-H at various times of pregnancy)

Tissue	Description of the treatment groups	Days pregnant	Theoretical daily intake (ng/person/day)		
			Estrone	Estradiol-17β	Testosterone
Fat	Unsynchronised controls	120	41	2	21
	Synchronised controls	120	57	2	27
	61 Days implanted	120	15	4	40
	Synchronised controls	180	137	3	37
	61 Days implanted	180	65	7	51
	Synchronised controls	240	136	3	33
	61 Days implanted	240	246	7	62
Muscle	Unsynchronised controls	120	46	3	74
	Synchronised controls	120	52	4	69
	61 Days implanted	120	18	7	99
	Synchronised controls	180	127	7	94
	61 Days implanted	180	36	7	93
	Synchronised controls	240	167	11	126
	61 Days implanted	240	117	9	116
Liver	Unsynchronised controls	120	2	5	4
	Synchronised controls	120	1	5	5
	61 Days implanted	120	1	2	4
	Synchronised controls	180	9	24	12
	61 Days implanted	180	2	3	6
	Synchronised controls	240	16	109	27
	61 Days implanted	240	2	15	9
Kidney	Unsynchronised controls	120	4	6	104
	Synchronised controls	120	3	5	71
	61 Days implanted	120	1	2	90
	Synchronised controls	180	8	13	140
	61 Days implanted	180	3	7	88
	Synchronised controls	240	7	15	191
	61 Days implanted	240	12	18	139
Theoretical Maximum Daily Intake (TMDI)	Unsynchronised controls	120	93	16	203
	Synchronised controls	120	113	16	172
	61 Days implanted	120	34	15	233
	Synchronised controls	180	280	48	282
	61 Days implanted	180	107	24	237
	Synchronised controls	240	326	139	377
	61 Days implanted	240	377	49	326

Table 51 calculates the theoretical daily intake resulting from the consumption of steers/heifers implanted with STEER-oid/HEIFER-oid. Tables 52- 56 summarise the intake calculations performed on the basis of the results obtained in studies with animals of both sexes and different ages/production classes, which had been treated with COMPUDOSE. Tables 63-66 summarise the intake calculations performed on the basis of the results obtained in studies with animals of both sexes and different ages/production classes, which had been treated with Finaplix, Revalor, Torelor and Revalor/Implix

Table 51. Theoretical daily intakes of estradiol-17β and progesterone from the tissues of steers/heifers implanted with STEER-oid/HEIFER-oid.

Days after implantation	Daily intake (ng/person/day)								
	Steers								
	Estradiol-17β			Progesterone			Testosterone		
	Muscle	Fat	TMDI	Muscle	Fat	TMDI	Muscle	Fat	TMDI
Control	13	8	21	186	113	299	ND	ND	ND
15	17	8	25	246	129	375	ND	ND	ND
	Heifers								
	Estradiol-17β			Progesterone			Testosterone		
	Muscle	Fat	TMDI	Muscle	Fat	TMDI	Muscle	Fat	TMDI
Control	11	5	16	ND	ND	ND	31	12	43
15	13	6	18	ND	ND	ND	34	14	48

ND = not determined

Table 52. Theoretical Dietary Intakes from the tissues of steers implanted with COMPUDOSE implants

Tissue	Treatment group (Study APH216A)	Concentration (median) (ng/kg)		Daily Intake (ng/person/day)	
		Estrone	Estradiol-17β	Estrone	Estradiol-17β
Fat	Control	10.9	6.3	0.5	0.3
	Implanted	39.5	20.9	2.0	1.0
Kidney	Control	9.1	9.4	0.5	0.5
	Implanted	24.2	29.9	1.2	1.5
Liver	Control	8.3	5.9	0.8	0.6
	Implanted	15.1	9.5	1.5	1.0
Muscle	Control	8.4	10.4	2.5	3.1
	Implanted.	9.1	7.4	2.7	2.2
TMDI	Control			4.4	4.5
	Implanted.			7.4	5.7

Table 53. Theoretical Dietary Intakes from the tissues of heifers implanted with COMPUDOSE implants*

Tissue	Treatment group	Concentration (mean) (ng/kg)		Daily Intake (ng/person/day)	
		Estrone	Estradiol-17β	Estrone	Estradiol-17β
Fat	Control	5.3	5.6	0.3	0.3
	Implanted	9.9	9.3	0.5	0.5
Kidney	Control	6.5	8.5	0.3	0.4
	Implanted	10.2	26.3	0.5	1.3
Liver	Control	7.5	8.2	0.8	0.8
	Implanted	6.7	7.3	0.7	0.7
Muscle	Control	5.9	7.1	1.8	2.1
	Implanted.	6.4	5.8	1.9	1.7
TMDI	Control			3.1	3.7
	Implanted.			3.6	4.3

• = Calculations are based on average residue levels because individual animal data were not available and, therefore, median values could not be calculated.

Table 54. Theoretical Dietary Intakes from the tissues of bull calves implanted with COMPUDOSE implants

Tissue	Treatment group	Concentration (median) (ng/kg)		Daily Intake (ng/person/day)	
		Estrone	Estradiol-17β	Estrone	Estradiol-17β
Fat	Control	13	5	0.7	0.3
	Implanted	23	40	1.2	2.0
Kidney	Control	8	11	0.4	0.5
	Implanted	35	56	1.7	2.8
Liver	Control	9	14	0.9	1.4
	Implanted	23	31	2.3	3.1
Muscle	Control	7	5	2.1	1.5
	Implanted.	11	19	3.4	5.6
TMDI	Control			4	4
	Implanted.			9	14

Table 55. Theoretical Dietary Intakes from the tissues of bulls implanted with COMPUDOSE implants

Tissue	Treatment group	Concentration (median) (ng/kg)		Daily Intake (ng/person/day)	
		Estrone	Estradiol-17β	Estrone	Estradiol-17β
Fat	Control	13.6	9.0	0.7	0.5
	Implanted	33.6	19.1	1.7	1.0
Kidney	Control	8.5	9.7	0.4	0.5
	Implanted	13.6	20.9	0.7	1.0
Liver	Control	5.5	8.7	0.5	0.9
	Implanted	6.1	15.3	0.6	1.5
Muscle	Control	7.2	5.0	2.2	1.5
	Implanted.	6.8	8.0	2.0	2.4
TMDI	Control			3.8	3.3
	Implanted.			5.0	5.9

Table 56. Theoretical Dietary Intakes from the tissues of Zebu steers implanted with COMPUDOSE implants

Tissue	Treatment group	Concentration (median) (ng/kg)		Daily Intake (ng/person/day)	
		Estrone	Estradiol-17β	Estrone	Estradiol-17β
Fat	Control	5.3	5.0	0.3	0.3
	Implanted	7.7	6.7	0.4	0.3
Kidney	Control				
	Implanted				
Liver	Control	8.0	8.1	0.8	0.8
	Implanted	11	14	1.1	1.4
Muscle	Control	17	8.6	5.0	2.6
	Implanted.	10	5.5	2.9	1.6
TMDI	Control			6.1	3.6
	Implanted.			4.4	3.4

Table 57. Theoretical daily intakes from the tissues of heifers treated with FINAPLIX

Day after implantation	Definition of the estrogen	Theoretical daily intake (ng/person/day)				
		Muscle	Liver	Kidney	Fat	TMDI
15	Free Estradiol-17β	4.7	1.7	0.63	2.4	9.4
	Conjugated Estradiol-17β	3.3	8.9	1.3	0.70	14
	Free Estrone		3.0		1.7	4.6
	Total Estrogens	11	14	1.9	4.5	31
30	Free Estradiol-17β	6.8	2.7	0.58	2.0	12
	Conjugated Estradiol-17β	6.6	8.8	0.78	0.73	17
	Free Estrone		2.5		2.7	5.2
	Total Estrogens	13	13	1.6	5.6	33
60	Free Estradiol-17β	2.1	2.3	1.0	1.3	6.7
	Conjugated Estradiol-17β	0.90	4.0	0.88	0.65	6.4
	Free Estrone		2.2		1.7	3.9
	Total Estrogens	2.9	8.7	1.9	3.7	17
75	Free Estradiol-17β	2.9	1.9	1.5	0.40	6.6
	Conjugated Estradiol-17β	3.5	2.4	2.0		7.8
	Free Estrone		1.8		1.9	3.7
	Total Estrogens	8.6	6.3	3.8	2.3	21

Table 58. Theoretical daily intakes of estradiol-17β from the tissues of animals implanted with Revalor

Sex of animal	Sample	Theoretical Intakes (ng/person/day)				
		Muscle	Fat	Liver	Kidney	TMDI
Heifers	Control	0.15	0.01	0.00	0.85	1.0
	Day 15	0.93	1.2	0.41	1.8	4.3
	Day 30	2.1	1.3	1.1	2.0	6.6
Steers	Control	0.63	0.33	0.00	1.1	2.0
	Day 15	1.26	0.90	0.09	1.1	3.4
	Day 30	1.38	0.71	0.05	0.97	3.1

Table 59. Theoretical daily intakes from the tissues of steers implanted with TORELOR A: Effects of a single implantation

Days after the last implantation	Daily intake (ng/person/day)						TMDI (ng/person/day)	
	Estrone		Estradiol-17β				Estrone	Estradiol-17β
	liver	fat	muscle	liver	kidney	fat		
Control	1.7	1.4	9.5	5.4	2.8	1.4	3.1	19.1
15	5.4	3.5	12	34	7.0	3.5	8.9	56
30	6.3	4.6	14	38	8.6	4.6	11	66
60	19	4.4	14	5	5.3	4.4	23	29
75	1.8	3.7	11	5.6	8.1	3.7	5.5	28
B: Effect of a second implantation								
60	2.2	2.7	9.3	30	13	6.6	4.9	59
60+15	1.8	3.3	8.4	14	10	5.3	5.0	38
60+30	2.7	4.6	38	18	19	15	7.3	89
60+60	1.8	3.5	36	11	11	8.2	5.3	67

Table 60. Theoretical intakes of hormones resulting from the consumption of tissues of animals treated with REVALOR/IMPLIX

			Estradiol-17α	Estradiol-17β					Progesterone					Testosterone				
	Group	Day	Liver	Muscle	Liver	Kidney	Fat	TMDI	Muscle	Liver	Kidney	Fat	TMDI	Muscle	Liver	Kidney	Fat	TMDI
Male Animals	Control	30	57	2.2	3.2	0.65	0.59	6.6	270	75	203	80	628					
		80	69	6.1		0.46	1.2	7.7	123	186	51	133	493					
	Revalor only	65	111	28	14	7.7	4.8	55										
		80	132	35	16	8.9	3	63										
		100	67	34	11	8.6	2.5	55										
	Implix BM/ Revalor	15	95	11	15	4.3	4.6	35	182	60	50	270	562					
		30	68	14	7.6	6.3	1.9	30	179	92	140	326	737					
		50	82	18	19	6.7	4.8	49	232	77	70	433	812					
		65	130	34	18	10	8.0	70	80	2025	79	777	2961					
		80	128	32	21	13	6.1	72	145	63	61	347	616					
		100	233	52	23	13	10	97	150	44	73	400	667					
Female Animals	Control	30	60	1.7	3.2	0.85	0.76	6.5						1.83	11	4.8	1.11	19
		80	59	4.4	2.3	0.93	0.94	8.6						123	186	51	133	493
	Revalor only	65	88	25	8.0	7.2	0.81	41										
		80	111	30	15	9.9	6.5	61										
	Implix BM/ Revalor	15	179	32	51	12	3.8	99						108	20	29	51	208
		30	70	27	7.3	8.5	8.6	51						74	6.6	28	63	172
		50	70	27	9.5	7.3	6.0	49						68	7.1	26	38	138
		65	172	47	27	8.2	7.0	89						56	7.9	35	29	129
		80	156	47	15	23	6.6	91						54	7.2	32	36	129

APPRAISAL

The results of the residue studies were statistically evaluated. The distributions of the residues were described by a number of characteristics including the mean, standard deviation, geometric mean and median. The assumption of normal distribution of the hormone concentrations could not be defended. Taking into consideration that tissue concentrations were sometimes below the limits of detection (LOD) or the limits of quantification (LOQ) of the methods, the median was the most stable and convenient parameter to provide an estimate of a central tendency of the data without excluding any individual result or making specific assumptions on substitute values for results like "below the limit of quantification". Appropriate median values (see below) were therefore also used as the basis for the calculation of theoretical daily intakes.

The objective of the intake calculations was to obtain conservative estimates of the theoretically possible excess dietary intakes of preferential eaters of meat that could be attributed to the approved uses of the products reviewed. The calculations were, therefore, performed in the following stepwise manner:

For every given time point of every study the median hormone concentrations found in tissues of control animals and of treated animals were multiplied with the respective daily consumption figures for "meat" conventionally used by JECFA (300 g muscle, 100g liver, 50 g kidney, and 50 g fat per person per day). The median value of a residue or contaminant in food is the appropriate value to be used if lifetime dietary exposure was to be assessed. However, the procedure used here, is not directly comparable to the ones used in the cases of other substances for which the Committee had set MRLs and were the MRLs are used as an estimate of the concentrations of a given substance in a food commodity. The present approach was followed because no numerical MRLs were established by the Committee.

- The results obtained in this way for muscle, liver, kidney and fat were summed up to calculate a figure for the total intake for 500 g of "meat".

- If data for several time points after implantation were available, the time points with the highest values were used. By doing this it was taken into consideration that no withdrawal period had been established for the use of any approved product. If the highest values did not coincide in time for all hormones, the time point with the highest results for estrogen intakes was selected. This was done in order to ensure a very conservative approach to the intake estimates of these compounds for which safety margins are somewhat lower than for the other two compounds. The effects of this selection on the estimates obtained for the other hormones were negligible. For the purposes of this report these figures are referred to as "Theoretical Maximum Daily Intakes"

- In order to estimate the defined excess intakes, the TMDI calculated for the concurrent untreated control population was subtracted from the figure obtained using the corresponding data of implanted animals.

Table 61 summarizes the final results of all relevant intake calculations. It also provides information on excess intakes of estrogens, the most relevant group of residues.

For total estrogens the highest excess intakes calculated in this way were in the order of 30-50 ng/person/day (see Table 61 Synovex in heifer in conjunction with comment 1 to the table and Finaplex in heifers in conjunction with comment 5 to the table). This range of excess intakes is less than 2% of the ADI for estradiol-17β established by the Committee for a 60 kg person. For certain experimental studies carried out with experimental combinations the resulting excess intakes were more than twice as high (about 4% of the ADI) if compared with the approved uses.

For progesterone the highest excess intake of the parent compound (which represents the only relevant hormonally active residue) was below 500 ng/person/day for the approved uses of this hormone (see Table 61, Synovex in calves). This excess intake corresponds to approximately 0.003% of the ADI established by the Committee for a 60 kg person.

For testosterone the highest excess intake (see Table 61, Synovex in heifers) for the free hormone was approximately 60 ng/person/day for all approved uses. This intake is approximately 0.2 % of the ADI established by the Committee for a 60kg person. The not precisely known intakes of possible relevant metabolites could theoretically be of the same order of magnitude.

The Committee noted that the hormone concentrations found in individual populations of treated animals - despite the fact that they typically were statistically significantly higher than the corresponding values of the concurrent controls - were well within the physiological range of these substances in bovine animals. In addition, the calculated excess

intakes contributed only a small additional hormonal burden to the background dietary intakes resulting from the consumption of other normal foods of both animal and plant origin.

Taking into consideration that the available data on the identity and concentrations of residues of the approved veterinary drugs in animal tissues indicate a wide margin of safety for consumption of residues in food when the products are used according to good practice in the use of veterinary drug, the Committee concluded that there would be no need to specify numerical MRLs for the three hormones and recommended "MRLs not specified" in bovine tissues. It is, however, recommended to keep the total intake of estrogenic residues resulting from the use of any approved hormonal product below the above calculated excess intakes.

Table 61. Calculations of excess TMDI from bovine animals treated with estradiol-17β, progesterone and testosterone

Product	Animals	Comments	Description of the treatment of the animals	Theoretical Maximum Daily Intakes [nanograms/person/day]					
				E_1	E_2-17α	E_2-17β	Excess E_1+E_2-β	P	T
Synovex-S (E_2-b+P)	Steers	1	**Control animals**	**1.0**		**0.5**		**190**	
			Animals slaughtered 15 days after implantation	2.0		6.3	6.8	254	
Synovex H (E_2-b+T-p)	Heifers	1	**Control animals**	**1.4**		**1.5**			**17**
			Animals slaughtered 15 days after implantation	3.9		15	16		70
Synovex-C (E_2-b+T) Synovex-H (E_2-b+T-p)	Calves a) female	1	**Control animals, slaughtered on day 61**	**1.1**		**3.5**		**22**	
			Control animals, slaughtered on day 119	**1.2**		**2.2**		**53**	
			Control animals, slaughtered on day 240	**0.8**		**1.7**			**13**
			Control animals, slaughtered on day 301	**1.4**		**4.4**			**22**
			Control animals, slaughtered on day 329	**0.7**		**2.1**			**22**
			Control animals, slaughtered on day 360	**2.0**		**3.0**			**51**
			implanted day 0; slaughtered on day119	3.0		3.7	3.3	520	
Synovex-C (E_2-b+T-p) Synovex S (E_2-b+P)	Calves b) castrated males		**Control animals, slaughtered on day 61**	**0.8**		**0.7**		**501**	
			Control animals, slaughtered on day 119	**0.4**		**0.5**		**552**	
			Control animals, slaughtered on day 240	**1.1**		**1.2**		**669**	
			Control animals, slaughtered on day 301	**0.5**		**0.9**		**421**	
			Control animals, slaughtered on day 329	**1.2**		**0.7**		**536**	
			Control animals, slaughtered on day 360	**0.8**		**1.0**		**1170**	
			implanted on days 0, 118, 240; slaughtered on day 301	3.7		11	13.3	540	
Synovex H (E_2-b+T-p)	Pregnant heifers	2	**120 days pregnant , unsynchronized controls**	**93**		**16**			**203**
			120 days pregnant , synchronized controls	**113**		**16**			**172**
			120 days pregnant , 61 days implanted	34		15	-80		233
			180 days pregnant , synchronized controls	**280**		**48**			**282**
			180 days pregnant , 61 days implanted	107		24	-197		237
			240 days pregnant , synchronized controls	**326**		**139**			**377**
			240 days pregnant , 61 days implanted	377		49	-39		326
Steer-oid (E_2 + P)	Steers	3	**Control animals**			**21**		**299**	
			Animals slaughtered 15 days after implantation			25	4	375	
Heifer-oid (E_2 +T-p)	Heifers		**Control animals**			**16**			**43**
			Animals slaughtered 15 days after implantation			18	2		48
Compudose (E_2)	Steers	4	**Control animals**	**4.4**		**4.5**			
			Animals implanted 70-180 days	7.4		5.7	4.2		

Table 61. Calculations of excess TMDI from bovine animals treated with estradiol-17β, progesterone and testosterone

Product	Animals	Comments	Description of the treatment of the animals	Theoretical Maximum Daily Intakes [nanograms/person/day]					
				E_1	E_2-17α	E_2-17β	Excess $E_1+E_2-\beta$	P	T
Synovex-S (E_2-b+P)	Steers	1	**Control animals**	**1.0**		**0.5**		**190**	
			Animals slaughtered 15 days after implantation	2.0		6.3	6.8	254	
Synovex H (E_2-b+T-p)	Heifers	1	**Control animals**	**1.4**		**1.5**			**17**
			Animals slaughtered 15 days after implantation	3.9		15	16		70
Synovex-C (E_2-b+T) Synovex-H (E_2-b+T-p)	Calves a) female	1	**Control animals, slaughtered on day 61**	**1.1**		**3.5**		**22**	
			Control animals, slaughtered on day 119	**1.2**		**2.2**		**53**	
			Control animals, slaughtered on day 240	**0.8**		**1.7**			**13**
			Control animals, slaughtered on day 301	**1.4**		**4.4**			**22**
			Control animals, slaughtered on day 329	**0.7**		**2.1**			**22**
			Control animals, slaughtered on day 360	**2.0**		**3.0**			**51**
			implanted day 0; slaughtered on day119	3.0		3.7	3.3	520	
Synovex-C (E_2-b+T-p) Synovex S (E_2-b+P)	Calves b) castrated males		**Control animals, slaughtered on day 61**	**0.8**		**0.7**		**501**	
			Control animals, slaughtered on day 119	**0.4**		**0.5**		**552**	
			Control animals, slaughtered on day 240	**1.1**		**1.2**		**669**	
			Control animals, slaughtered on day 301	**0.5**		**0.9**		**421**	
			Control animals, slaughtered on day 329	**1.2**		**0.7**		**536**	
			Control animals, slaughtered on day 360	**0.8**		**1.0**		**1170**	
			implanted on days 0, 118, 240; slaughtered on day 301	3.7		11	13.3	540	
Synovex H (E_2-b+T-p)	Pregnant heifers	2	**120 days pregnant , unsynchronized controls**	**93**		**16**			**203**
			120 days pregnant , synchronized controls	**113**		**16**			**172**
			120 days pregnant , 61 days implanted	34		15	-80		233
			180 days pregnant , synchronized controls	**280**		**48**			**282**
			180 days pregnant , 61 days implanted	107		24	-197		237
			240 days pregnant , synchronized controls	**326**		**139**			**377**
			240 days pregnant , 61 days implanted	377		49	-39		326
Steer-oid (E_2 + P)	Steers	3	**Control animals**			**21**		**299**	
			Animals slaughtered 15 days after implantation			25	4	375	
Heifer-oid (E_2 +T-p)	Heifers		**Control animals**			**16**			**43**
			Animals slaughtered 15 days after implantation			18	2		48
Compudose (E_2)	Steers	4	**Control animals**	**4.4**		**4.5**			
			Animals implanted 70-180 days	7.4		5.7	4.2		

Table 62. Percent contributions of the TMDI in the four animal tissues

Substance implanted	Testosterone propionate		Estradiol benzoate				Progesterone	
Animals:	Heifers		Heifers		Steers		Steers	
Fraction of total labeled residue	Free	Conjugated	Free	Conjugated	Free	Conjugated	Free	Conjugated
	Contribution (%) to the TMDI							
Muscle	6.7	5.3	5.3	3.7	5.9	3.1	3.0	1
Liver	29.9	38.1	36.5	13.5	21.5	28.5	17.2	15.8
Kidney	3.8	4.2	9.9	18.1	9.9	18.1	26.5	22.5
Fat	11.0	1.0	12.2	0.8	11.6	1.4	13.2	0.8
Total	**51.4**	**48.6**	**63.9**	**36.1**	**48.9**	**51.1**	**59.9**	**40.1**

2 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle, liver, kidney and fat. The fractions of the conjugated hormones were not determined. A correction of the data is not possible due to a lack of relevant information. However, in view of the well established significant reduction in the TMDI as a consequence of implantation of pregnant heifers such a correction is apparently unnecessary.

3 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle and fat. The given figures most likely greatly underestimate the "true" TMDI 's. No information was available to correct these estimates.

4 The method used includes the extraction and de-conjugation of conjugates. The estimated intake figures, therefore, represent total parent compound and can be used as they are given in the table.

5 The free and conjugated fractions of estradiol-17β were determined in all tissues. However, estrone (free fraction only) was only determined in liver and fat. The "true" TMDI 's for estrogens, therefore, could well be 50% higher than the values given in the table. Data on which a more precise estimate of a correction factor could be based were not available. The Torelor study represents research work and does not reflect an approved use.

6 From the method description it appears that conjugates are not included in the determination of the residues. In view of the effects of trenbolone/estradiol combinations on estrogen concentrations seen in other studies, it cannot be excluded that the data given in the table significantly underestimate the "true" TMDI for estrogens.

7 No description of the analytical method was given in the report. The values for estradiol-17α are based on concentrations found in liver only. The study represents research work and does not reflect an approved use.

REFERENCES

Arts, C.J.M., van Baak, M.J., Huisman, J. and van Weerden, E.J. (1986a). Residue studies in heifers implanted with Finaplix ®. ILOB Report 560.TNO Wageningen. FDA/CVM INAD file 4216, Vol. 1, p. 2.

Arts, C.J.M., van Baak, M.J., Huisman, J. and van Weerden, E.J. (1986b). Residue studies in steers implanted with Torelor ®. ILOB report 561a. TNO Wageningen, FDA/CVM INAD file 4216, Vol. 2, p. 72.

Arts, C.J.M., van Baak, M.J., de Jong, J. and van Weerden, E.J. (1986c). Residue studies in steers after a bi-sequential implantation with Torelor ®. ILOB-report 572, TNO Wageningen. FDA/CVM INAD file 4216, Vol. 4, p. 649.

Clemson University (undated) Tissue residue study in heifers implanted with 180 mg trenbolone acetate/18 mg estradiol. In-Life portion of study: Southwest Bio-Labs; tissue assays Clemson University. FDA/CVM NADA file 140-992, Vol. 1, p.103, Vol. 11, p. 722, Vol. 17, p. 268.

Decker, O.D. and Turner, L.G. (undated). Estrogen tissue levels in bulls following treatment with estradiol controlled-release implants. Study AAC8410. Lilly Research Laboratories. Information provided to FAO for Review by JECFA, Vol. 1, p. 254.

FAO (a). Information provided to FAO for Review by JECFA, Vol. 1, p .21.

FAO (b). Determination of 17β-estradiol, estrone, and their sulfate and glucuronide conjugates in cattle tisssues. Information provided to FAO for Review by JECFA, Vol. 1, p. 359.

FAO (c). Estradiol-17β and estrone in liver, kidney, lean and kidney fat of steers treated with estradiol controlled release implants (Determination of indicator tissue and compound). Study APH-216 A, B, E. Information provided to FAO for Review by JECFA, Vol. 1, p. 113.

FDA/CVM NADA file 9-576, Vol. 3, p. 199. .Metabolism of Synovex-S® (Estradiol-17β benzoate - progesterone combination) in the steer and Synovex-H® (Estradiol-17β benzoate - testosterone combination) in the heifer.

FDA/CVM NADA file 9-576, Vol. 4, p. 211. .Preliminary characterization of radioactivity in tissues obtained at slaughter from steers and heifers implanted in the ear with ^{14}C-labeled Synovex-S and Synovex-H, respectively.

FDA/CVM NADA file 9-576, Vol. 4, p. 262. Characterization of radioactivity in tissues obtained at slaughter from steers and heifers implanted in the ear with ^{14}C-labeled SYNOVEX-S and SYNOVEX-H, respectively: further characterization of radioactivity in tissues of steer C.

FDA/CVM NADA file 9-576 Vol. 4, p. 318. Metabolism of Synovex-S® (estradiol-17β benzoate - progesterone combination) in the steer and Synovex-H® (estradiol-17β benzoate - testosterone combination) in the heifer.

Frank, R., Basson, R.P. and Wagner, J.F. (undated, a). Determination of empirical cumulative distribution functions of endogenous estradiol-17β and estrone levels in slaughter steers and effect of location, breed, carcass weight and federal grade on the endogenous levels. Final report on 306 animals. Study APH-227. Information provided to FAO for Review by JECFA, Vol. 1, p. 95.

Frank, R., Basson, R.P. and Wagner J.F. (undated, b). Depletion of estradiol-17β and estrone from kidney fat of steers following removal of the estradiol controlled release implant. Study APH-216 C. Lilly Research Laboratories. Information provided to FAO for Review by JECFA, Vol. 1, p. 138.

Henricks, D.M. and Frank, R. (undated). Estradiol-17α in liver, kidney and kidney fat tissues of steers treated with estradiol controlled release implants. Study APH-216E. Information provided to FAO for Review by JECFA, Vol. 1, p. 159

Kushinsky, S. and Duffy, J. (1979). Continuation of studies of the development, validation and application of radioimmunologic procedures for the determination of estrone, estradiol-17β, progesterone and testosterone in bovine fat, muscle, Kidney, and liver. Progress Report, January 16 - October 15, 1979 by Stanley Kushinsky and Jane Duffy, Institute of Biological Sciences, Syntex Research. Vol. 5, p. 204, FDA/CVM NADA file 9-576.

Kushinsky, S. and Duffy, J. (undated). Continuation of studies on the development, validation and application of radioimmunologic procedures for the determination of estrone, estradiol-17β, progesterone and testosterone in bovine fat, muscle, kidney, and fat. Stanley Kushinsky and Jane Duffy, Institute of Biological Sciences, Syntex Research. FDA/CVM NADA files 9-576 Vol. 15, p. 006 and 11-427, Vol. 14, p. 033, Vol. 15, p.315, Vol. 16, p. 002.

Kushinsky, S. and Duffy, J. (1982). Continuation of studies on the development, validation and application of radioimmunologic procedures for the determination of estrone, estradiol-17β, progesterone and testosterone in bovine fat, muscle, kidney and liver. Pregnant heifers treated with Synovex-H. Progress Report of April 6, 1982 by Stanley Kushinsky and Jane Duffy. Department of Analytical and Metabolic Chemistry, Institute of Pharmacology and Metabolism, Syntex Research. FDA/CVM NADA file 11-427, Vol. 16, p. 308

Kushinsky, S. and Duffy, J. (1983). Synovex calf study: concentrations of estradiol-17β, estrone, testosterone and progesterone in the edible tissues following a single implantation or sequential implantations of Synovex. Progress Report, May 5, 1983 by Stanley Kushinsky and Jane Duffy, Department of Analytical and Metabolic Chemistry, Institute of Pharmacology and Metabolism, Syntex Research. FDA/CVM NADA file 9-576, Vol. 23, p. 57.

Kushinsky, S. and Mirrasoul, M. (1979). Continuation of studies on the development, validation and application of radioimmunologic procedures for the determination of estrone, estradiol-17β and progesterone in bovine fat, muscle, kidney and liver. Progress Report of January 15, 1979 by Stanley Kushinsky and Marilyn Mirrasoul, Institute of Biological Sciences, Syntex Research. FDA/CVM NADA file 11-427, Vol.5, p. 100.

Lee, G.J.-L- and Massey, I.J. (1989). Effect of re-implantation of Synovex S on the concentration of estradiol-17β and progesterone in the plasma of steers and the implications with regard to human food safety. Syntex Research., Study report 8902, August 31, FDA/CVM NADA file 9-576, Vol. 30, p. 180.

Lindsey, G.D., Sieck, R.F. and Wagner, J.F (undated, b). Zero withdrawal fat tissue estrone levels in steers treated with four times the recommended dosage of an estradiol controlled-release implant. Lilly Research Laboratories. Information provided to FAO for Review by JECFA, Vol. 1, p. 348.

Marcus, M. (undated). Letter of M. Marcus, New York Medical College to Ivy-Gene Corp. FDA/CVM NADA file 135-906, Vol. 1, p. 72.

Marcus, M., Meyes, E. and Kadi, R. (undated). Determination of tissue concentrations of estradiol and progesterone in "Steeroid" (Ivy-Reed) implanted steer and validation of radioimmunoassays., Department of Pathology, Lincoln Medical Center, New York Medical College. FDA/CVM NADA file 110-315, Vol. 2, p. 105.032.

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 Huntingdon Research Centre. FDA/CVM INAD file 4216, Vol. 3, p. 457. Concentrations of the steroid hormones were determined at the University of Liège (Belgium).

Sheldon, J.J., Kushinsky, S., Spires H.R. and Herschler, R.C. (1983). Tissue concentrations of estradiol-17β, estrone, testosterone and progesterone in calves implanted with Synovex® implants. Final Report (August 10, 1983). James J. Sheldon, investigator; report prepared by Stanley Kushinsky, Howard R. Spires, and Richard C. Herschler, Syntex Research. Vol. 23, p. 003, FDA/CVM NADA file 9-576.

Sieck, R.F, Frank, R., Turner, L.G., Basson, R.P. and Wagner J.F. (undated, a). Confirmation that a 24-hour withdrawal time is sufficient to allow estradiol-17β and estrone levels in kidney fat of Compudose-implanted steers to recede to untreated normal population levels. Study S-AAC-79-12. Lilly Research Laboratories. Information provided to FAO for Review by JECFA, Vol. 1, p. 177.

Sieck, R.F. and Turner, L.G. (1981). Interlaboratory validation of method AM-AA-CA-R015-AA-755 "Determination of estrone in cattle kidney fat" Study S-AAC-80-33. Information provided to FAO for Review by JECFA, Vol. 5, p. 26.

Sieck, R.F. and Turner, L.G. (undated). Estrogen tissue residues in Brazilian Zebu steers following treatment with estradiol controlled-release implants. Study T1EBL8004. Information provided to FAO for Review by JECFA, Vol. 1, p. 280.

Sieck, R.F, Turner, L.G., Wagner, J.F. and Basson, R.P. (undated, b). Estrogen tissue levels in heifer cattle following treatment with estradiol controlled-release implant. Study S-AAC-82-14. R.F. Sieck, L.G. Turner, J.F. Wagner, R.P. Basson. Information provided to FAO for Review by JECFA, Vol. 1, p.197 and Vol. 5, p.136

Sieck, R.F, Turner, L.G., Matsuaka,T. and Basson, R.P. (undated, c). Estrogen tissue levels in veal calves following treatment with estradiol-17β controlled-release implants. Study S-AAC-80-43. Lilly Research Laboratories. Information provided to FAO for Review by JECFA, Vol. 1, p. 218.

Weiert Velle (1976). Endogenous Anabolic Agents in Farm Animals., in "Environmental Quality and Safety, Anabolic Agents in Animal Production" , eds. F. Coulston and F. Corte, Suppl. Vol. V., pp 171-180. Thieme, Stuttgart

Wray, M.I., (undated) Blood levels of estradiol and trenbolone acetate in steers implanted with Revalor S/G and grazed on summer pasture. Horton Feedlot and Research Centre. Study code HR-247. FDA/CVM NADA file 140-897, Vol. 22, p. 1372.