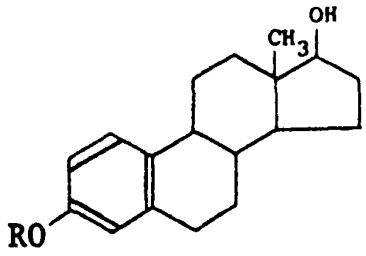
ESTRADIOL

IDENTITY

Chemical name: estra-1,3,5(10)-triene-3,17β-diol

Synonyms: estradiol-17β

Structural formula:



R = H(estradiol)
R = OCC_6H_5(estradiol benzoate)

Molecular formula: $C_{18}H_{24}O_2$ (estradiol), $C_{25}H_{28}O_3$ (estradiol benzoate)

Molecular weight: 272.37 (estradiol), 376.50 (estradiol benzoate)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

	estradiol	estradiol benzoate
Appearance:	white crystals or crystalline powder	crystals
m.p.:	173-179°C	191-196°C
Optical rotation:	$[\alpha]_D^{25}$ = +76 to +83° (dioxane)	+58 to +63° (c=2 in dioxane)
UV_{max}:	225, 280 nm	

(Windholz, 1983)

Technical active ingredients

USP Grade	Estradiol benzoate:	97.0-103.0 percent $C_{25}H_{28}O_3$
	Estradiol :	97.0-103.0 percent $C_{18}H_{24}O_2$

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Estradiol, alone or in combination with other hormonally-active substances, is given to animals to improve their rate of weight gain and feed efficiency. The most likely explanation of this effect is that the pituitary is stimulated to produce increased quantities of growth hormone. Administration is by sub-cutaneous implant in the ear. The ear, along with any residual drug, is discarded at slaughter.

Dosages:

Compudose (24 mg or 45 mg estradiol) = cattle
Heifer-oid (20 mg estradiol benzoate + 200 mg testosterone propionate) = heifers
Implix BF (20 mg estradiol + 200 mg testosterone) = heifers
Implix BM (20 mg estradiol + 200 mg progesterone) = steers
Steer-oid (20 mg estradiol benzoate and 200 mg progesterone) = steers
Synovex C (10 mg estradiol benzoate and 100 mg progesterone) = calves
Synovex H (20 mg estradiol benzoate and 200 mg testosterone propionate) = heifers
Synovex S (20 mg estradiol benzoate and 200 mg progesterone) = steers
Torelor (40 mg estradiol + 200 mg trenbolone acetate) = steers

RADIOLABELED RESIDUE STUDIES

General

Estradiol benzoate is rapidly hydrolyzed in the target animal to estradiol-17β. In the circulatory system of the animal, the estradiol-17β derived from the implant is indistinguishable from the endogenous estradiol-17β. (Hoffman and Evers, 1986)

Cattle

After administration of radiolabeled estradiol-17β to calves, radioactivity in urine consisted primarily of estradiol-17α with much less amounts of estrone. Both compounds were present as conjugates as well. Radioactivity in feces included primarily estradiol-17α as well as estradiol-17β and estrone, each in the non-conjugated form. (Ivie, et al., 1986)

After administration of estradiol benzoate, the major metabolites found in muscle were estradiol-17α (38-70% of extracted radioactivity) and estrone (17-45%). The pattern of metabolites in fat was similar to that for muscle. The highest residues were found in kidney and liver. The major estrogenic metabolites in kidney were estradiol-17α, estradiol-17α-glucuronide, estradiol-17β and estrone. In liver, the major metabolite could not be identified (40% of the extracted radioactivity). Estradiol-17β, estrone, estriol, and glucuronides accounted for the remaining radioactivity. (Dunn, et al., 1977)

The nature of the unidentified polar metabolites from livers of steers was investigated in another study using radiolabeled estradiol-17β. The major polar metabolite was the β-D-glucopyranoside of estradiol-17α. The 3-β-D-glucosiduronate of estradiol-17α, and other 17-glycosides of estradiol-17α and estradiol-17β were also characterized. (Rao, et al., 1979)

RESIDUE STUDIES

Steers (Compudose)

Fifty-three steers were implanted for 70-180 days with 24 mg estradiol-17β (E2β) controlled-release implants. Various withdrawal times were observed prior to slaughter. The implants released approximately 70 mcg of E2β/day. Tissue samples collected from untreated steers were also analyzed. Measurements of E2β and estrone (E1) were made using an RIA method. The limit of detection was approximately 5 pg/g for both compounds. The concentrations of E2β and E1 in edible tissues of control and treated steers at various withholding times are given in Table I. [Frank, et al., undated (a)]

Table I. E2β and E1 Mean Tissue Concentrations in Steers Implanted with Compudose (ng/kg)

Withdrawal Period	Tissue			
	Muscle	Liver	Kidney	Fat
E2β				
Control	5.8 ± 3.3	4.0 ± 1.7	6.7 ± 3.4	6.8 ± 4.2
0 Hr	3.4 ± 1.7	10.0 ± 9.1	21.1 ± 6.4	15.3 ± 4.6
12 "	3.3 ± 1.6	4.0 ± 1.6	7.5 ± 3.7	8.8 ± 5.3
24 "	4.0 ± 1.0	5.0 ± 1.0	7.5 ± 3.1	7.1 ± 2.1
E1				
Control	4.8 ± 2.7	6.5 ± 3.4	7.9 ± 3.8	10.5 ± 6.4
0 Hr	10.4 ± 6.7	8.8 ± 6.4	19.2 ± 6.6	34.8 ±11.1
12 "	9.5 ± 4.3	4.1 ± 1.7	10.3 ± 4.9	25.3 ± 9.3
24 "	4.0 ± 1.0	4.7 ± 0.6	7.1 ± 0.5	14.3 ± 2.3

Steers (Compudose)

Seventy-seven steers were implanted with controlled-release implants containing 24 mg of estradiol to study the depletion of estradiol and estrone from kidney fat after implant removal. Total treatment time ranged from 90 to 147 days. Kidney fat was obtained by biopsy. The assay procedure employed an RIA with a detection limit of approximately 5 pg/g. The mean estrogen residues found at different withdrawal times is given in Table II: [Frank, et al., undated (b)]

Table II. Residues of Estrogens in Kidney Fat (ng/kg)

Withdrawal Time (hr)	N*	E1	E2β
0	4	23.5	6.5
12	12	10.5	3.9
24	23	7.8	3.4
36	29	5.6	3.4
72	9	10.6	7.1
Control	19	11.1	7.3

*N = Number of tissue samples (animals) in group

Non-pregnant Heifers (Compudose)

A study was conducted to determine the estrogen levels in tissues of non-pregnant heifers treated with 24 mg estradiol controlled-release implants.

Heifers weighing approximately 800 lbs. were used in the study. The heifers were divided into two groups - one control group and one treated group. The treated animals were slaughtered 84 days after implanting; tissues were analyzed for E2β and E1 by RIA. The residue levels measured are given in Table III:

Table III. E2β and E1 Mean Tissue Concentrations in Non-Pregnant Heifers Treated with Compudose (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
E2β				
Control	7.1 ± 3.3	8.2 ± 4.0	8.5 ± 4.0	5.6 ± 1.1
Treated	5.8 ± 1.1	7.3 ± 2.5	26.3 ±11.9	9.3 ± 4.7
E1				
Control	5.9 ± 1.8	7.5 ± 4.8	6.4 ± 3.0	5.3 ± 1.1
Treated	6.4 ± 2.2	6.7 ± 2.4	10.2 ± 6.4	9.9 ± 6.7

Estrogen levels in liver and muscle of treated animals were not significantly increased as compared to control tissues. Residue levels were significantly higher in kidney and fat. [Sieck, et al., undated (b)]

Veal calves (Compudose)

Forty veal calves (mean weight 94 lbs.) were implanted with 24 mg of estradiol-17β. Twenty of the treated animals were slaughtered after 56 days with no removal of the implant. The remaining treated animals had the implants removed after 56 days and were slaughtered 24 hours after implant removal. Tissue samples were analyzed for E2β and E1 by an RIA with a limit of detection of 5 pg/g. Mean concentrations levels for the various tissues and treatment groups are given in Table IV:

Table IV. E2β and E1 Mean Tissue Concentrations Veal Calves Treated with Compudose at 0 and 24 hr Withdrawal (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
E2β				
Control	6.8 ± 3.4	14.7 ± 5.8	10.6 ± 5.3	11.2 ± 14.9
0 hr	18.4 ± 5.6	32.0 ± 11.2	57.0 ± 18.1	38.5 ± 10.9
24 "	6.1 ± 1.7	16.0 ± 8.3	10.9 ± 4.2	7.1 ± 2.8
E1				
Control	10.8 ± 8.7	9.1 ± 3.6	7.8 ± 4.6	13.9 ± 4.8
0 hr	11.7 ± 5.7	22.3 ± 8.8	34.7 ± 9.8	24.6 ± 12.0
24 "	8.0 ± 4.1	9.2 ± 4.4	9.6 ± 3.7	10.4 ± 4.8

Residue levels of estrogens were significantly increased in treated animals that did not have the implants removed prior to slaughter. [Sieck, et al., undated (a)]

Bulls (Compudose)

Twelve bulls weighing approximately 850 lbs. were used in this study. Six animals were implanted with 24 mg of estradiol-17β. After 63 days both the treated and control animals were sacrificed and tissue samples were taken for analysis of E1 and E2β by an RIA procedure. Mean residue levels are given in Table V.

Table V. E2β and E1 Mean Tissue Concentrations in Bulls Treated with Compudose (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
E2β				
Control	6.3 ± 2.0	8.5 ± 3.2	10.0 ± 3.4	9.1 ± 2.0
Treated	8.5 ± 2.7	16.6 ± 12.4	19.9 ± 6.2	20.2 ± 6.2
E1				
Control	7.7 ± 3.1	6.0 ± 1.2	8.7 ± 3.0	15.3 ± 8.2
Treated	6.8 ± 1.9	6.9 ± 2.6	15.1 ± 5.4	29.6 ± 10.4

There is a statistically significant increase in estrone and estradiol levels in kidney and kidney fat tissues in implanted bulls as compared to tissues from control bulls. (Decker et al., 1985)

Cattle (Synovex-S and Synovex-H)

Residues of estradiol-17β and estrone were measured in edible tissues from steers implanted with Synovex-S, pregnant and non-pregnant heifers implanted with Synovex-H, unimplanted steers, and unimplanted pregnant and non-pregnant heifers. Residues were measured by using an RIA with a limit of detection in the low ng/kg range. Mean residue levels in tissues at various times after implantation are given in Tables VI-VIII. (Kushinsky, 1983)

Table VI. E2β and E1 Mean Tissue Concentrations in Steers Implanted with SYNOVEX-S (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
E2β*				
Control	0.84 ± 0.30	0.91 ± 0.42	1.57 ± 1.61	1.82 ± 1.02
15-day	9.70 ± 3.2	5.36 ± 2.05	13.7 ± 3.02	41.4 ± 8.53
30-day	8.28 ± 5.14	6.75 ± 4.93	9.45 ± 5.40	28.6 ± 21.8
61-day	7.29 ± 2.51	4.52 ± 5.48	6.45 ± 1.89	38.6 ± 11.9
E1*				
Control	1.60 ± 0.65	0.66 ± 0.24	1.02 ± 0.49	8.47 ± 5.10
15-day	2.12 ± 0.59	2.04 ± 0.84	4.44 ± 1.37	20.3 ± 9.15
30-day	1.99 ± 0.85	1.79 ± 0.94	2.56 ± 1.23	14.4 ± 7.71
61-day	2.60 ± 1.47	2.10 ± 3.57	2.06 ± 0.46	26.0 ± 12.93

* All concentrations except E1 in muscle are significantly ($P<.001$) higher than controls.

Table VII. E2β and E1 Mean Tissue Concentrations in Heifers Implanted with SYNOVEX-H (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
E2β*				
Control	5.54 ± 8.77	1.54 ± 1.12	2.89 ± 2.50	13.4 ± 14.1
30-day	33.2 ± 19.6	23.1 ± 36.6	23.5 ± 11.8	86.7 ± 65.9
61-day	10.7 ± 5.14	3.2 ± 2.36	9.83 ± 8.98	49.3 ± 30.8
E1*				
Control	2.51 ± 1.05	1.70 ± 1.15	1.42 ± 0.56	10.9 ± 5.94
30-day	6.43 ± 3.68	3.69 ± 3.36	5.88 ± 2.27	45.4 ± 29.4
61-day	3.98 ± 1.45	1.49 ± 0.76	3.56 ± 2.02	27.8 ± 13.8

* All concentrations except E1 in 61-day muscle are significantly ($P<.01$) higher than controls.

Table VIII. E2β and E1 Mean Tissue Concentrations in Pregnant Heifers Implanted With SYNOVEX-H (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
120-Day Gestation				
E2β				
Control**	13.3 ± 5.21	82.5 ± 75.9	118 ± 58.6	48.1 ± 19.8
61-Day	30.6 ± 11.2*	18.7 ± 7.38*	62.3± 44.8	82.9 ± 37.6*
E1				
Control	156 ± 79.3	18.2 ± 15.1	85.3 ± 69.4	1283 ± 885
61-Day	83.1± 67.8	5.55 ± 2.2*	29.7 ± 23.1	421 ± 352*
180-Day Gestation				
E2β				
Control	27.3 ± 14.3	380 ± 280	230 ± 99.9	71.5 ± 37.2
61-Day	24.7 ± 7.9	50.4± 33.6*	126 ± 58.2	123 ± 58.9
E1				
Control	482 ± 301	115 ± 82.6	166 ± 94	2717 ± 1259
61-Day	167 ± 108*	23.4± 13.7*	59.4± 36.6*	1896 ± 1290
240-Day Gestation				
E2β				
Control	32.7 ± 16.1	1027 ± 365	274 ± 84.8	67.5 ± 34.6
61-Day	26.3 ± 8.2	198 ± 173*	318 ± 107	136 ± 27.2*
E1				
Control	523 ± 240	145 ± 55.4	142 ± 41.2	2786 ± 1497
61-Day	353 ± 109	34.5± 41.4*	214 ± 67.0	4614 ± 1513

* Significantly different from synchronized controls.
** Controls were synchronized with fenprostalene.

The results of these studies show that there are small but statistically significant residues of estradiol and estrone in some tissues of steers treated with Synovex-S and heifers treated with Synovex-H. In general, the residues of estrogens found in fat exceed those found in the other tissues. The

concentrations of endogenous estrogens are also greater in fat than in the other edible tissues. The average concentrations of estrone plus estradiol-17β in fat obtained from animals implanted for 61 days with Synovex-S exceed those present in fat from control animals by 54.3 ng/kg. The comparable value for fat from non-pregnant heifers is 52.8 ng/kg.

The concentrations of estrone and estradiol-17β found in all tissues from pregnant heifers exceed those found in tissues from non-pregnant heifers. In comparison with the concentrations of endogenous hormones found in tissues from pregnant animals, all hormonal residues of Synovex-S and Synovex-H are exceedingly small, regardless of how soon after implantation the tissues are obtained. For 9 of the 12 statistically significant differences shown in Table VIII for pregnant heifers, the concentrations were higher in tissues from the control animals.

Steers (Torelor)

Twenty-four black and white Friesian steers weighing approximately 400 lbs were implanted with Torelor (40 mg estradiol-17β + 200 mg trenbolone acetate). Six additional steers were used as controls. Six treated steers were slaughtered at four post-implantation times: 15, 30, 60 and 75 days. Free and conjugated E2β and E1 were determined by a radioimmunoassay with detection limits of 70 pg/g (free E2β), 75 pg/g (conjugated E2β) and 27 pg/g (E1). The results are summarized in Table IX. (Arts, et al., 1986)

Calves (Implix BM or Implix BF)

Nine male and nine female Friesian calves were implanted with either Implix BM (males) (20 mg estradiol-17β + 200 mg progesterone) or Implix BF (female) (20 mg estradiol-17β + 200 mg testosterone). Groups of six (three males and three females) were sacrificed at 15, 30 and 50 days post-implantation. The results for E2β (free + conjugated) with a detection limit of 70 pg/g are summarized in Table X. (Roberts and Cameron, 1986)

Table IX. E2β and E1 Mean Tissue Concentrations in Steers Treated with Torelor (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
E2β-free				
Control	23 ± 12	21 ± 7	16**	14**
15-Day	40 ± 31	40 ± 25*	53 ± 10	119 ± 17
30-Day	39 ± 24	62 ± 48*	53 ± 10	122 ± 41
60-Day	37 ± 15	25 ± 9*	51 ± 29	126 ± 47
75-Day	28 ± 17	20 ± 5*	74 ± 43	84 ± 60
E2β-conjugated				
Control	9**	35 ± 9	41 ± 4	10**
15-Day**	14**	296 ± 253	99 ± 31	14**
30-Day	20**	306 ± 191	117 ± 36	13**
60-Day	8**	295 ± 333	111 ± 140	24**
75-Day	11**	99 ± 137*	133 ± 129	9**
E1				
Control	-	16**	-	24**
15-Day	-	53 ± 37	-	69 ± 14
30-Day	-	59 ± 25	-	93 ± 12
60-Day	-	57 ± 42	-	85 ± 16
75-Day	-	36 ± 47	-	69 ± 26

** Means below the detection limit were not statistically analyzed.
* Not significantly (P>0.05) different from control.

Table X. E2β Mean Tissue Concentrations in Steers (Heifers) Treated With Implix BM (Implix BF) (ng/kg)

	Tissue			
Steer	Muscle	Liver	Kidney	Fat
Control	7 ± 2	32 ± 11	13 ± 5	12 ± 5
15 Day	36 ± 6	154 ± 60	85 ± 19	92 ± 76
30 Day	48 ± 22	76 ± 23	125 ± 58	38 ± 45
50 Day	60 ± 13	193 ± 49	134 ± 28	96 ± 76
Heifer				
Control	5 ± 1	32 ± 9	17 ± 3	15 ± 3
15-Day	106 ± 22	512 ± 263	249 ± 115	75 ± 63
30-Day	88 ± 6	73 ± 20	170 ± 63	171 ± 24
50-Day	89 ± 28	95 ± 106	146 ± 104	119 ± 35

METHODS OF RESIDUE ANALYSIS

General

The majority of methods for hormones are radioimmunoassays. Some of these methods were developed by the sponsors of the drugs to determine the level of naturally occurring steriods in the bovine and to elucidate the pharmacokinetics and residue pattern of specific steriod drugs in the bovine. These methods were not intended for regulatory use. Specific results from the method of two sponsors are discussed here because it is important to understand why different methods yield different results on essentially the same tissues.

Both Syntex and Elanco provide data on the level of 17β-estradiol in control steer tissue (all values in ng/kg).

	Elanco	Syntex
Fat	6.8 (kidney fat)	1.82
Muscle	5.8	0.84
Liver	4.0	0.91
Kidney	6.7	1.57

Although these levels appear quite different, each is at or close to the stated sensitivity of the method (approximately 5 ng/kg for Elanco and 1-2 ng/kg for Syntex). Factors affecting assay sensitivity include: affinity and specificity of the antibody employed (currently difficult to control fully or standardize), extent to which the assay conditions are optimized, procedural losses and size of sample analyzed. Considering the purpose for which the Elanco and Syntex methods were intended and the magnitude of the residues found by each of the sponsors for their respective products, both methods are judged to be suitable for the analysis of residues of estradiol in the primary edible tissues. Consequently, it does not appear advisable to recommend a single method for the assay of estradiol, should monitoring be necessary. The performance characteristics and a quality assurance program would be a more appropriate recommendation.

APPRAISAL

Methods of analysis based on immunological techniques are the only methods readily available to measure estradiol-17β and its metabolites in tissue of cattle treated with subcutaneous implants of the drug. Using these methods, an increase in estradiol-17β and estrone concentrations in almost all edible tissues compared to control has been demonstrated using a variety of implant formulations. However, the increase is small (ng/kg) when compared to the background levels in control animals and the random variation in the methods. In addition to the random variations in the methods at these low levels (ng/kg), the methods are sufficiently unique that they do not measure a specific chemical moiety with the same accuracy. Therefore, one must be careful in comparing residue concentrations from more than one controlled experiment.

Unless the implant is removed, the increased levels of estradiol-17β and estrone persist for extended periods of time. However, these small increases in estrogen residue levels determined in treated steers, heifers and calves are smaller than the variations seen in cycling heifers and much smaller than the large increases in estrogen residue levels seen in pregnant heifers.

Estradiol-17β occurs naturally in all mammals including human beings. The daily production rate in humans is given in Table XI (Farber and Arcos, 1983). The levels of estradiol-17β in meat are extremely small compared to these daily production rates. For example, the average amounts of estradiol-17β produced daily in men and in pregnant women are respectively, about 15,000 and several million times the amount contained in a 500 g portion of meat from an animal treated with the hormone according to good animal husbandry practice. Even in prepubertal boys, the most sensitive segment of the population, the amount of estradiol-17β produced daily is a thousand times the amount derived from progesterone of 500 g of treated meat.

Table XI. Estradiol Production Rates in Humans

	µg/day
Women	
Follilcular phase	445
Luteal phase	270
Late pregnancy	37,800
Postmenopausal	8
Prepubertal girls	31
Men	
Adult	48
Prepubertal boys	6

REFERENCES

Arts, C.J.M., van Baak, M.J., Huisman, I.J., and van Weerden Ir. E.J. (1986). Residue studies in steers implanted with TORELOR. Unpublished report IL)B-report 561a. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Decker, O.D. and Turner, L.G. (1985). Estrogen tissue levels in bulls following treatment with estradiol controlled-release implants. Unpublilshed study No AAC8410 from Lilly Research Laboratories, Greenfield, IN, USA. Submitted to FAO by Elanco Products, Co., Indianapolis, IN, USA.

Dunn, T.G., Kaltenbach, C.C., Koritnik, D.R., Turner, D.L., and Niswender, G.D. (1977). Metabolites of estradiol-17 and estradiol-17-3-benzoate in bovine tissues. J. Anim. Sci. 46, 659-673.

Farber, T.M. and Arcos, M. (1983). A regulatory approach to the use of anabolic agents In: Meissonnier, E. (ed.) Anabolics in Animal Production, Office International des Epizooties, Paris, pp. 289-296.

Frank, R., Basson, R.P., and Wagner, J.F. [Undated (a)]. Estradiol-17β and estrone in liver, kidney, lean and kidney fat tissues of steers treated with estradiol controlled release implants. Unpublished report No. APH-216 A,B,E from Lilly Research Laboratories, Greenfield, IN, USA. Submitted to FAO by Elanco Products Co., Indianapolis, IN, USA.

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. Unpublished report No. APH-216 C from Lilly Research Laboratories, Greenfield, IN, USA. Submitted to FAO by Elanco Products Co., Indianapolis, IN, USA.

Hobkirk, R. and Nilsen, M. (1971). Metabolism of 17-estradiol to 17-estradiol-3- glucosiduronate and 17-estradiol-17glucosiduronate by the normal human female. J. Clin. Endocr. 3., 779-785.

Hoffmann, B. and Evers, P. (1986) Anabolic agents with sex hormone-like activities: Problems of residues. In: Rico, A.G. (ed.), Drug Residues in Animals, Academic Press, New York, pp. 111-146.

Ivie, G.W., Christopher, R.J., Munger, C.E., and Coppock, C.E. (1986). Fate and residues of [4-14C]estradiol-17 after intramuscular injection into holstein steer calves. J. Anim. Sci. 62, 681-690.

Kushinsky, S. (1983). Safety aspects of the use of cattle implants containing natural steriods. Presented at: International Symposium on the Safety Evaluation of Animal Drug Residues, West Berlin. Submitted to FAO by Syntex (U.S.A.) Inc., Palo Alto, CA, USA.

Roberts, N.L. and Cameron, D.M. (1986) Steroid levels in tissues of veal calves following implantation with Implix BM/BF and/or Revalor lactose. Unpublished report No. RSL/686 from Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Rao, P.N., Purdy, R.H., Williams, M.C., Moore, P.H. Jr., Goldzieher, J.W., and Layne, D.S. (1979). Metabolites of estradiol-17 in bovine liver: identification of the 17-D-glucopyranoside of estradiol-17&. J. Steriod Biochem. 10, 179-185.

Sieck, R.F., Turner, L.G., Matsuoka, T., and Basson, R.P. [Undated (a)]. Estrogen tissue levels in veal calves following treatment with estradiol-17 controlled-release implants. Unpublished study No S-AAC-80-43 from Lilly Research Laboratories, Greenfield, IN, USA. Submitted to FAO by Elanco Products Co., Indianapolis, IN, USA.

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. Unpublished report No S_AAC-82-14 from Lilly Research Laboratories, Greenfield, IN, USA. Submitted to FAO by Elanco Products Co., Indianapolis, IN, USA.

Windholz, M., ed. (1983). The Merck Index 10th edition. Merck and Co., Rahway, N.J.

****