

I. GENERAL INFORMATION

A. File Number

NADA 138-612

B. Sponsor

Roussel-Uclaf

C. Proprietary Name

Finaplix

D. Established Name

Trenbolone acetate

E. Dosage Form

Pellet

F. Dosage Regimen

FOR HEIFERS

One implant for heifers contains 200 mg trenbolone acetate. Each heifer implant contains 10 pellets of 20 mg trenbolone acetate in each pellet which make up the dosage of Finaplix® and are contained in one division of a multiple cartridge. Ten doses are in each cartridge. The cartridge is designed to be used with a special implant gun. The implant is placed under the skin on the posterior aspect of the ear by means of an implant gun. This product is to be used in heifers only during the last 63 days prior to slaughter.

FOR STEERS

One implant for steers contains 140 mg trenbolone acetate. Each steer implant contains 7 pellets of 20 mg trenbolone acetate in each pellet. The same type multiple dose cartridge and implant gun are used for both the steer and heifer products. For continued effectiveness in steers this product should be reimplanted once after 63 days.

G. Route of Administration

The product is supplied as an ear implant for cattle.

H. Species / Class

Cattle

I. Indication

The product is an implanted anabolic agent with slow release of trenbolone acetate which increases rate of weight gain and improves feed efficiency in growing finishing heifers and improves feed efficiency in growing finishing steers.

II. EFFECTIVENESS

A. Pivotal Studies

The new animal drug application on which trenbolone acetate is based contains adequate and well-controlled studies demonstrating the effectiveness of the new animal drug for the indications for use as given in Item 2 above.

The pivotal studies are dose titration studies in which the parameters measured are the same parameters as are measured in clinical (field) studies. The pivotal studies have been conducted using a uniform protocol for the studies in heifers and a uniform protocol for the studies in steers so that the results of the studies may be pooled and summarized separately for heifers and steers. The studies were conducted in the major beef producing areas of the United States.

Name and address of Investigators:

Heifer Studies:

Mr. Preston Grover, Colorado Beef Producers, Division of Continental Grain,
Lamar, CO

Dr. Dallas Horton, Horton Feedlot and Research Center, Wellington, CO

Dr. Werner Bergen, Department of Animal Sciences, Michigan State University, E.
Lansing, MI

Dr. T. W. Perry, Department of Animal Sciences, Purdue University, W. Lafayette,
IN

Steer Studies:

Dr. Dallas Horton, Wellington, CO

Preston Grover, Lamar, CO

Dr. Marvin Sharp, Sharp Veterinary Research, Vernon, TX

John Combs, Southwest Idaho Research Center, Caldwell, ID

The purpose of the studies was to determine the dose response for trenbolone acetate implants on average daily gain and feed efficiency of growing finishing heifers and steers. The test animals were crossbred animals of European breeds. There were eight animals per pen in all studies. Each treatment was replicated 4 or 5 times in the heifer and steer studies. The heifer and steers weighed approximately 800 (363 kg) and 740 (336 kg) pounds, respectively, when the studies were initiated. A total of 1,280 steers and heifers were treated with trenbolone acetate in these dose titration studies. Four steer and four heifer studies were conducted.

The trenbolone acetate was given via ear implants. The implants were placed subcutaneously on the backside of the mid-ear. Each dose was made up of the appropriate number of pellets, with each pellet containing 20 mg trenbolone acetate. The control cattle were not implanted. The dosages given in the heifer studies were 0, 140, 200 and 300 mg. The dosages for the steer studies were 0, 40, 80, 140 and 200 mg. The heifers were implanted once with an implant period

of 63 days. The steers were implanted at day 0 and again at day 63. The duration of the steer studies was 126 days.

A randomized complete block design was used for all studies and the data were pooled by Analysis of Variance to determine the significance of the effect of trenbolone acetate implants on average daily gain and feed efficiency. For the heifer data there was a significant ($P < 0.05$) dose effect on average daily gain and feed efficiency with the maximum effect on both parameters at a dosage of 200 mgs (table 1). For the steer data there was a significant ($P < 0.05$) improvement in feed efficiency with the best response at 140 mgs (table 2).

These data are sufficient to support the claims and dosage as outlined in sections 2 and 3 above and no adverse reactions which affected animal safety were reported during the effectiveness studies described above.

(Eds. note: The following table consists of 5 columns.)

TABLE 1. SUMMARY OF DOSE RESPONSE IN HEIFER STUDIES -
IMPLANT PERIOD 63 DAYS

Average Daily Gain (kg)

	Trenbolone acetate (mg)			
Location	0	140	200	300
Indiana	1.07	1.12	1.21	1.12
Michigan	1.09	1.04	1.14	1.15
Colorado 1	1.62	1.75	1.74	1.67
Colorado 2	1.24	1.23	1.32	1.28
Average	1.26	1.29	1.35	1.31

Feed Efficiency (F/G)

	Trenbolone acetate (mg)			
Location	0	140	200	300
Indiana	6.53	6.08	5.56	6.03
Michigan	7.27	7.58	7.04	6.99
Colorado 1	5.84	5.21	5.29	5.46
Colorado 2	7.02	6.79	6.36	6.33
Average	6.67	6.42	6.06	6.20

TABLE 2. SUMMARY OF DOSE RESPONSE IN STEER STUDIES
IMPLANT PERIOD 126 DAYS

Average Daily Gain (kg)

	Trenbolone acetate (mg)				
Location	0	40	80	140	200
Colorado 1	1.37	1.37	1.47	1.49	1.47
Colorado 2	1.24	1.32	1.30	1.28	1.33
Texas	1.42	1.43	1.30	1.44	1.34
Idaho	1.16	1.20	1.22	1.25	1.26
Average	1.30	1.33	1.32	1.37	1.35

Feed Efficiency (F/G)

	Trenbolone acetate (mg)				
Location	0	40	80	140	200
Colorado 1	8.27	8.24	7.84	7.60	7.79
Colorado 2	5.38	5.22	5.36	5.23	5.22
Texas	7.42	7.19	7.24	7.03	7.30
Idaho	7.93	7.61	7.64	7.39	7.22
Average	7.25	7.07	7.02	6.81	6.88

III. TARGET ANIMAL SAFETY

A. Study 1

A drug tolerance study was conducted by Professor H. Zucker at the Institute for Physiology, School of Veterinary Medicine, University of Munich, Munich, West Germany. The purpose of the study was to determine the effect of an exaggerated dose. The test animals were young heifer calves weighing approximately 140 pounds. There were three calves in each treatment group. The dosages used were control, 140 mg trenbolone acetate and 3500 mg trenbolone acetate. The 3500 mg dose is 17.5 times the recommended dosage of 200 mg trenbolone acetate. Ten (10) weeks after implantation the calves were sacrificed. The parameters measured were clinical observations, gross necropsy, histology, clinical chemistry, hematology and organ

weights. The high level (3500 mg) of trenbolone acetate caused abnormal development of the clitoris and reduction in the weight of the thymus. A reduction in ovary weights was seen in all treated calves. The calves tolerated this exaggerated dosage with minimal adverse effects noted. These effects included proliferation of uterine glandular tissues in some of the treated animals. This effect would be predicted based on the hormonal activity of the compound.

B. Pivotal Study

A target Animal Safety study was conducted by Dr. N.L. Roberts, Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. The purpose of the study was to assess the safety to beef cattle of trenbolone acetate given subcutaneously as an ear implant. Twenty-four (eight per group) yearling cattle weighing approximately 500 pounds at the initiation of the study were given the following dosages of trenbolone acetate: control (no implant), 200 mg and 1000 mg per animal. There were four (4) steers and four (4) heifers in each of the three treatment groups. The cattle were reimplanted after 63 days and the study was terminated after 126 days. The parameters measured were clinical signs, animal health, body weights, clinical biochemistry, and carcass grading.

It was concluded that the treatment of beef cattle with trenbolone acetate by subcutaneous implantation on two occasions (with an interval of 63 days) at 200 mg or 1000 mg per head, did not result in any adverse effects on clinical health. Bodyweight gains were increased in comparison with controls. Carcass weights were heavier for the treated cattle and there were no adverse effects on carcass quality. Haematological and biochemical parameters remained within normal limits.

IV. HUMAN FOOD SAFETY

A. Toxicity Studies

A rat oral toxicity study to determine reproductive effects was conducted by Dr. Brian Hunter, Huntingdon Research Centre, Huntingdon, England. The object of the study was to assess the toxicity of trenbolone acetate when administered in the diets of rats which were derived from dams treated with trenbolone acetate during pre-mating, mating, gestation and lactation periods. The study was divided into a "reproductive phase" dealing with the reproductive performance of F0 generation covering a period from inception to weaning of the litters of the F0 generation. The "main phase" of the study was with the F1 generation and covered a 13 week period after weaning. In the reproductive phase there were 24 rats (12 males and 12 females) per treatment group for a total of 120 rats. During the main phase there were 50 rats (25 males and 25 females) per treatment group for a total of 250 rats. The dietary levels of trenbolone acetate were 0, 1, 2, 5, and 10 ppm.

The results of the study were as follows:

Reproductive phase:

During the pre-mating period a slightly lower body weight gain associated with a lower food intake was recorded for adult males treated at 10 ppm. There were no treatment related effects on the reproductive performance of animals treated with

trenbolone acetate and no treatment related differences in litter size, litter weight, pup size or mortality.

Main phase:

At the 10 ppm level, a higher food intake with an associated higher body weight gain in females was recorded. A lower body weight gain in males was recorded at the 10 ppm level. The 10 ppm level also produced higher serum alkaline phosphatase levels, higher absolute and relative liver weights in females 4 weeks after weaning only, lower seminal vesicle weights 4 weeks after weaning (not associated with any morphological change) and at week 13 the seminal vesicle weights were similar to those of the controls. At the 5 ppm level, a higher food intake with an associated higher body weight gain in females was observed. The 5 ppm level produced a lower weight gain in males. The 5 ppm level also produced lower relative seminal vesicle weights 4 weeks after weaning (not associated with any morphological change) but at week 13 the seminal vesicle weights were similar to those of the controls. At 2 ppm a higher food intake with an associated higher body weight gain in females was observed, a lower weight gain was observed in males. At 1 ppm a lower weight gain was observed in males.

The treatments produced an increased weight gain in females receiving 2, 5, or 10 ppm associated with an increased food intake. Although a lower weight gain was produced in all treated males, in the absence of a dose relationship this finding must be regarded as equivocal. Based on the above findings, it was concluded that 1 ppm probably represents a no effect level for trenbolone acetate in rats.

Trenbolone acetate was fed to mice in a long term chronic feeding study by Dr. Brian Hunter, Huntington Research Centre, Huntington, England. The purpose of the study was to assess the potential tumorigenicity of trenbolone acetate on mice. 640 mice were started on the study and the female mice were fed for up to 96 weeks and the male mice were fed for up to 104 weeks. Trenbolone acetate was mixed into the diet and fed at levels of 0, 0.5, 1.0, 10.0, and 100.0 ppm. There were 128 mice (64 males and 64 females) in each treatment group at the beginning of the experiment. There were no significant differences in all parameters measured in this study except for:

inhibition of ovulation and uteri with a histological appearance consistent with diestrus among females killed after 13 weeks of treatment.

macroscopic lesions associated with the following histopathological changes:

1. significant increases in hepatic proliferative lesions (neoplasia and hyperplasia) in males and females and an increase in hepatocyte vacuolation in males.
2. a marginal increase in nephritis in females.
3. reduction in splenic haemopoietic activity in females
4. cystically dilated ducts and/or abscess of the female preputial glands.

The significant increase in the macroscopic lesions was treatment related with the highest incidence at the highest dose (100 ppm) level. The Cancer Assessment Committee of the Center for Veterinary Medicine examined this chronic study in the mouse. The Committee concluded that the effects observed were a manifestation of

the well documented hormonal effects of some naturally occurring and synthetic anabolic steroids in a recognized target tissue. Support for this opinion was also derived from data which suggest that trenbolone acetate is not mutagenic (see summary of mutagenic study reports).

The potential toxicity of prolonged administration of trenbolone acetate to rats following in utero exposure was studied by Dr. Brian Hunter, Huntington Research Centre, Huntington, England. The purpose of the study was to evaluate the potential toxicity and tumorigenicity of trenbolone acetate when administered in the diet of rats derived from dams that have themselves received trenbolone acetate. Seven hundred twenty rats were randomly assigned to one of six treatments for the in utero phase of the study. Six hundred rats were used for the main phase of the study (lifetime study of F1 generation). These rats were randomly selected from the litters produced in the in utero phase. Trenbolone acetate was mixed into the diets of the rats and fed continuously at levels of 0, 0.5, 1.0, 4.0, 16.0, and 50.0 ppm. During the reproductive phase of the study there were no overt signs of a reaction to treatment with trenbolone acetate exhibited by parent animals. Rats of the F0 generation receiving trenbolone acetate at a level of 1 ppm or greater appeared to show a general treatment related impairment of reproductive performance. The numbers of pups per litter were reduced in comparison with the controls among females receiving 16 or 50 ppm. At day 4 the anogenital distance of pups of the 50 ppm group was noted to be similar for both males and females, thereby making sexual distinction difficult. The major findings at termination of the main phase of the study were in the sex related characteristics. These changes were seen in the majority of females receiving 50 or 16 ppm and these changes included coarse male-like fur, perineal hair loss, prominent pudendum, small ovaries, uterus pale and containing viscous fluid and cervix not palpable. Trenbolone acetate was noted to have a emasculating effect on treated males. The incidence and severity of these findings was treatment related. The incidence of certain aging changes appeared to be reduced in females receiving 50 ppm. Male rats receiving 50 ppm appeared to have an increase of small adrenals and small pituitaries in comparison with the controls. Significantly low prostate, testes, and kidney weights for males receiving 50 ppm were considered to be the result of treatment with trenbolone acetate. Prostate and testes weight of males receiving 16 ppm were also significantly low in comparison with the controls. Females receiving 50 ppm had significantly low adrenal and ovary weights. Significant microscopic findings included absent corpora lutea, inflammation and modification of the vagina, inflammation, luminal dilation and decreased endometrial thickness of the uterus, clitoral enlargement and development of clitoral bone in the females. Testicular, prostatic and vesicular atrophic changes were noted in the male rats. In the 50 ppm treatment group the female rats had increased urinary bladder calculi, increased incidence of ground glass hepatocytes in the liver, increased incidence of Harderianisation of the lachrymal glands, decreased incidence of mammary fibrous adenomas, and decreased incidence of pituitary adenomas. This study was also reviewed by the Cancer Assessment Committee of the Center for Veterinary Medicine. On the basis of direct and ancillary evidence, the committee concluded that the increased incidence in pancreatic islet cell tumors are not the result of a carcinogenic effect of trenbolone acetate. Based on these chronic lifetime rat and mouse studies the Cancer Assessment Committee agreed that the application of a conservative safety factor to the non-observed hormonal effect as proposed in the Center for Veterinary Medicines hormone policy document was appropriate for the regulation of trenbolone acetate.

Therefore the results of the animal feeding studies indicated that the principal effects of trenbolone acetate were associated with the hormonal activity of this compound. The critical studies for assessment of human food safety, therefore, were related to the determination of the lowest level that would not produce hormonal effects in a female monkey model system.

Trenbolone acetate was studied in a preliminary oral toxicity study in *Cynomolgus* monkeys by Dr. Rodney Sortwell, Huntington Research Centre, Huntington, England. The object of the study was to obtain preliminary information relating to the toxicity of trenbolone acetate when administered by oral gavage to young adult *Cynomolgus* monkeys. The monkeys were dosed once daily for 8 weeks by oral gavage. Two pairs (1 male and 1 female in each pair) of monkeys received trenbolone acetate at levels of 0.375 mg/kg per day or 1.875 mg/kg per day. These two dosages for a 4 kg. monkey consuming 300 gms of dry diet per day would be equivalent to 5 or 25 ppm trenbolone acetate respectively. A third pair of animals acted as the controls. After a period of 8 weeks the monkeys were killed and subjected to macroscopic examination followed by microscopy of selected tissues. There were no mortalities during the course of the study and there were no treatment related effects on clinical signs, body weight or feed consumption. The males showed decreased prostate weights and increased seminal vesicles and testes weights.

A study to determine the hormonal no effect dose level for trenbolone acetate in the female Rhesus Macaque was conducted by Dr. David Hess at the Oregon Regional Primate Research Center, Beaverton, Oregon. The purpose of the study was to determine the hormonal no effect dosage of trenbolone acetate in a representative female primate. Trenbolone acetate was administered in the diet for 3 estrous cycles or a maximum of 122 days to 3 groups of 6 mature female monkeys at 60, 240 and 960 µg/day. Blood samples were obtained on a daily basis from all animals during a pretreatment menstrual cycle, at 3 day intervals during the first 2 treatment menstrual cycles and daily during the last treatment menstrual cycle or 30 days. Serum concentrations of estradiol, progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined by radioimmunoassay. The largest dose resulted in maximum average serum levels of 2.3 ng/ml of 17β-trenbolone and this dose may have inhibited gonadotropin secretion and ovarian function in 3 of 16 reproductive cycles. We conclude that trenbolone had no effect at the mid and low dose although the inhibitory effects of orally administered trenbolone on the reproductive parameters studied in these females were marginal, at least in comparison with progestational compounds. A conservative hormonal no effect level was established at 40 microgram per kg per day (240 microgram per day via the diet).

SUMMARY

The results of these animal feeding studies indicated that the principal effects of trenbolone acetate were associated with the hormonal activity of the compound. The critical study for assessment of human food safety therefore was related to the determination of the lowest level that would not produce hormonal effects in a female monkey model system. The hormonal effects of trenbolone acetate were as follows:

Impairment of reproductive performance in rats. Female rats had coarse male-like fur, perineal hair loss, prominent pudendum, small ovaries and cervix not palpable.

Male rats receiving high doses had an increased incidence of small adrenals, small pituitaries, and lower prostate, testes, and kidney weights. In the Rhesus monkey studies trenbolone acetate appeared to inhibit gonadotropin secretion and ovarian function. The suppression of ovulation and cyclical ovarian activity was noted in some studies. Based on these studies the hormonal no-effect level was established based on the pivotal study conducted in the Rhesus monkey. A conservative hormonal noeffect level was established at 40 microgram per kg per day (240 microgram per day via the diet).

B. Mutagenic Potential Study Reports

An Ames metabolic activation test to assess the potential mutagenic effect of trenbolone acetate, 17b estradiol and a 7:1 mixture of trenbolone with 17b estradiol was conducted by Dr. David Hossack, Huntington Research Centre, Huntington, England. In this in vitro assessment of the mutagenic properties of the test compounds histadine dependent auxotrophic mutants of Salmonella typhimurium were exposed to concentrations as high as 10,000 µg/plate of each of the test materials. The strains were tested on histadine deficient agar and the test material was diluted in dimethylsulfoxide. In a parallel series of plates, rat liver microsomal fraction was added to the agar to effect metabolic transformation of the test compound. After incubation for 72 hours at 37° C, the numbers of revertant (histadine independent) colonies were counted and compared with negative controls. No evidence of bacteriostatic activity was observed at the dose levels tested. Trenbolone acetate, 17b estradiol and the 7:1 mixture failed to show any evidence of mutagenic potential in this bacterial system.

A study was conducted by Dr. Margaret Richold at Huntington Research Centre, Huntington England to assess the mutagenic potential of 17 beta hydroxytrenbolone and 17 alpha hydroxytrenbolone when orally administered to rats. The mutagenic potential was assessed by examination of chromosome damage in somatic and germinal metaphase cells. The somatic cells were examined from the bone marrow and the germinal cells were spermatogonial cells. The natural hormones estradiol and testosterone were used as reference control substances. Mitomycin C, a known mutagen, was used as the positive control. The treatment compounds were administered to rats by intragastric intubation at a single dose of 100 mg/kg body weight in a single dose or the compounds were administered by intragastric intubation in four equal dosages separated by intervals of 24 hours at total doses of 100 and 200 mg/kg body weight. After administration of 17 beta hydroxytrenbolone, 17 alpha hydroxytrenbolone, estradiol or testosterone the group mean aberrant metaphase counts in both tissues were similar to the value observed with the control group. It was concluded that despite the apparent negative activity of 17B-hydroxy- and 17A-hydroxytrenbolone in the in vivo cytogenetic studies, study limitations of a single dose in vivo limited the interpretation of the negative findings.

A study was conducted by Dr. Margaret Richold at Huntington Research Centre, Huntington England to determine the mutagenic effect of 17 beta hydroxytrenbolone and 17 alpha hydroxytrenbolone on cultured human lymphocytes. The two compounds were tested in vitro to determine whether they cause chromosomal aberrations in cultured human lymphocytes. The test compounds produced chromosome aberrations comparable with the respective control values in the absence of metabolic activation or in the presence of metabolic activation. It was concluded that despite the apparent lack of treatment-related increase in

chromosome aberrations, the dosing schedule in vitro limited the interpretation of the negative findings.

The synthetic hormones 17 alpha hydroxytrenbolone and 17 beta hydroxytrenbolone and the natural hormones testosterone and estradiol were tested for mutagenic potency in an in vitro mouse lymphoma cell L5178Y/TK assay. This study was conducted by Dr. Margaret Richold at Huntington Research Centre, Huntington, England. With testosterone and estradiol, the criteria for a positive mutagenic response were not fulfilled. A weak but reproducible positive response was recorded in the mouse lymphoma gene mutation assay for both 17B-hydroxy- and 17A-hydroxytrenbolone.

The Ames metabolic activation test to assess the potential mutagenic effect of 17 alpha hydroxytrenbolone, 17 beta hydroxytrenbolone, and 17 alpha estradiol was conducted by Dr. Margaret Richold, Huntington Research Centre, Huntington, England. Five strains of Salmonella typhimurium were tested. 17 alpha hydroxytrenbolone was toxic towards the tester strains at 5,000 microgram per plate dose level, therefore 500 microgram per plate was chosen as the top dose level in the mutation test. 17 beta hydroxytrenbolone was slightly toxic towards the tester strains at 5,000 microgram per plate. Therefore, an intermediary dose level of 1500 microgram per plate was chosen as the top dose level in the mutation test. 17 alpha estradiol was not toxic toward the tester strains at the chosen dose levels, therefore 5,000 microgram per plate was chosen as the top dose level in the mutation test. No substantial increases in the revertant colony numbers of any of the 5 strains were observed following treatment with 17 alpha hydroxytrenbolone, 17 beta hydroxytrenbolone, or 17 alpha estradiol at any dose level either in the presence or absence of liver microsomal fraction. It was concluded that no evidence of mutagenic potential was seen in any of the compounds tested in this bacterial test system.

Dr. Margaret Richold at Huntington Research Centre, Huntington, England conducted a mutagenicity study with diethylstilbestrol, 17 beta hydroxy trenbolone, 17 beta estradiol and 17 alpha hydroxytrenbolone using the mouse lymphoma L5178Y cell mutation test. In this in vitro system it is possible to detect and quantitate forward mutation from a wild cell type. The four compounds were found to be toxic to the L5178Y cells: diethylstilbestrol between concentrations of 5 and 12.5 micrograms per ml, 17 beta hydroxytrenbolone between 15 and 65 microgram per ml, 17 beta estradiol at 90 microgram per ml and 17 alpha hydroxytrenbolone between 22.5 and 45 microgram per ml. Treatment of the cells with diethylstilbestrol and 17 beta hydroxytrenbolone produced twofold increases in mutation frequency at cell survival levels of greater than 20% relative to the control in each case. Both, 17 beta estradiol and 17 alpha hydroxytrenbolone also induced two-fold increases in mutation frequency, however, at cell survivals of less than 20% relative to the controls. The responses were dose-dependent, and the mutagenic activity in each case occurred in the presence of S9 metabolic activation. It is concluded that the assay results are indicative of the mutagenic potential of diethylstilbestrol, 17B-hydroxytrenbolone, 17B-estradiol, and 17A-hydroxytrenbolone.

In assessment of the mutagenic potential of 17 alpha hydroxytrenbolone in mammalian cells in vitro using the Chinese hamster ovary/HPRT locus assay was conducted by Dr. L. M. Henderson, Huntington Research Centre, Huntington, England. This study using a gene mutation assay in cultured mammalian cells was conducted to determine the potential mutagenicity of 17 alpha hydroxytrenbolone.

The compound was tested in the presence and absence of a source of supplementary metabolic activation on two separate occasions. This test determines the compounds ability to induce forward mutation at the functionally homozygous hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells. Treatment with 17 alpha hydroxytrenbolone induced dose-related cytotoxicity in CHO cells in both tests in the absence and presence of exogenous metabolic activation. It is concluded that the results were suggestive of a possible minor, though non-reproducible, mutagenic effect resulting from exposure of CHO cells to 17 alpha hydroxytrenbolone, in the absence of S9 metabolizing activity. The mean mutation frequencies induced by the two treatment conditions (100 and 150 mcg/ml) were elevated 3.33- and 2.22- fold, respectively. However, in view of the fact that (a) the reported results were not statistically significant, and (b) the mutagenic responses were not reproducible in an independent assay, it was concluded that the mutagenic activity caused by 17A- hydroxytrenbolone in this assay system was not considered substantial.

A BHK cell transformation assay on Trenbolone acetate was performed by Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. The results of the BHK 21 cell transformation assay indicated that under the assay conditions as performed BHK 21 cells were transformed by Trenbolone acetate both in the absence and presence of exogenous, S9 metabolizing activity. Furthermore, the induction of BHK 21 cell transformation was reproducible, occurring in two independent studies, both with and without S9 activation.

C. Reproduction Study Reports

A teratology study was conducted in the pregnant rat by Dr. Penny James, Huntington Research Center, Huntington, England. A preliminary study was conducted on 24 female rats given trenbolone acetate by intragastric intubation at levels of 0, 2.5, 5.0 or 10 mg/kg per day. The treatments were started on day 6 of pregnancy and continued daily up to and including day 15 of pregnancy. In this preliminary study the pregnant rats showed no overt adverse effects on maternal or litter parameters following treatment. For the main teratology study 80 female rats were treated with trenbolone acetate at levels of 0, 5, 10 and 20 mg/kg per day. Treatment was by intragastric intubation commencing on day 6 of pregnancy and continued daily up to and including day 15 of pregnancy. The animals were killed on day 20 of pregnancy, litter values determined were crown-rump length and anogenital distances measured and fetuses examined for skeletal and visceral anomalies. In this teratology study, treatment at 20 mg/kg per day was associated with hair loss in 15/20 animals, relaxed bodytone, slightly increased salivation and retardation of body weight gain. Animals receiving 10 mg/kg per day showed hair loss in 9/20 animals, slightly increased salivation and slight retardation of weight gain over the dosing period. Litter parameters as assessed by numbers of viable young, post implantation loss, litter and mean pup weight did not suggest any adverse effects of treatment. Embryonic and fetal development as assessed by the incidence of major malformations, minor visceral anomalies and skeletal variance did not appear adversely effected. There was a slightly higher incidence of minor skeletal anomalies at 20 mg/kg per day. The difference from control values was not statistically significant and there was no increase in any specific anomaly to account for the difference. For male fetuses preserved in Bouin's solution, there was an apparent slight dosage related trend for reduced anogenital distance. The difference at 20 mg/kg per day attained borderline statistical significance. No associated

decrease in fetal weight or crown-rump length was observed. Male fetuses preserved in alcohol did not show the same degree of differences in mean values although individual ranges in respect of high and low values were lower at 20 mg/kg per day than for controls. The mean anogenital distances for females were comparable for all groups. If the differences in male anogenital distance are considered real, then they must be of an extremely subtle nature since for fetuses preserved in Bouin's only one male in each test group had an anogenital distance which fell within the total individual range for females similarly preserved. Likewise only one male fetus preserved in alcohol at 5 mg/kg per day and 2 at 20 mg/kg per day fell within the total individual range for females similarly preserved.

The effect of trenbolone acetate on reproductive function of multiple generations in the rat was studied by Dr. Penny James at Huntington Research Centre, Huntington, England. Over 700 male and female rats were fed in this study to assess the effect of trenbolone acetate on growth and reproductive performance of the rat through two (2) consecutive generations. Trenbolone acetate was fed in the diet at concentrations of 0, 0.5, 3.0 and 18.0 ppm. The test diets were fed to F0 males for 9 weeks and F0 females for 2 two weeks prior to mating and then through to termination of the study. Two F1 generations were selected, reared and mated. One was treated continuously at the same dietary concentrations as the F0 generation and one was removed from exposure to trenbolone acetate at three weeks of age and remained untreated throughout. Treatment with trenbolone acetate at 18 ppm was associated with the following effects:

Generally higher weekly body weights effecting F0 and F1 treated males and females, and the females of the F1 untreated generation.

A depression in mean body weight gain during gestation affecting both matings of F0 generation.

Signs of coarseness of the hair coat and discoloration of the skin affecting F0 and treated F1 females.

Clitoral prominence in treated female F1 offspring and to a lesser extent in those where treatment was withdrawn at three weeks of age. Similar effects were observed in treated F2 offspring from treated F1 parents but not in offspring of the untreated F1 generation.

The presence of occlusive strands in the vagina and/or precocious incomplete vaginal opening affecting treated F1 pups and F2 pups from the treated F1 generation.

A delay in the occurrence of testicular descent affecting F2 pups from the treated F1 generation.

A marked reduction in pregnancy rate effecting the second mating the F0 generation and the treated F1 generation.

An increase in pre-coital time for the second mating of the F0 generation and the treated F1 generation.

A marginal extension of the duration of gestation affecting the F0 generation and the treated F1 generation.

A marked increase in the incidence of extended parturition in total litter loss in the treated F1 generation and a significant increase in the percentage of males per litter.

Effects on litter parameters, principally lower litter size and litter weight at birth, or 20 day sacrifice at both matings of the F0 and for the treated F1 generation and increased post implantation/pre-birth loss for the F0 and treated F1 generation.

At terminal autopsy an increase in the incidence of depressions in the fore stomach epithelium affecting F0 and F1 males was observed.

Among organ weights of adults consistent findings included a significant reduction in seminal vesicle/prostate weight in F0 and F1 treated males and an increase in mean ovary weight among F0 and F1 treated females.

Among organ weights of offspring at 6 weeks, F1 and F2 male pups showed a significant decrease in the weight of seminal vesicle/prostate, testes, and epididymides. F1 and F2 female pups showed reduction of adrenal weight.

The laboratory examinations relating to the second mating of the F0 generation (teratology phase) revealed a significant reduction in anogenital distance among male fetuses and a marginal increase in the incidence of skeletal variance.

The only apparent effects of treatment with trenbolone acetate at 0.5 ppm were:

Higher group mean body weights for males of the F0 generation.

A slight but not statistically significant delay in the mean age of vaginal opening of F1 pups and F2 pups of the F1 treated generation; subsequent mating performance and resulting litter parameters were comparable with those of controls.

Among organ weights of the offspring at 6 weeks treated F1 and F2 male pups from the treated parents showed a statistically significant decrease in seminal vesicles/prostate weight with the F2 males additionally showing a significant decrease of weight of epididymides.

Trenbolone acetate exerted a marked effect at 18 ppm and some effect at 3 ppm when considered in terms of reproductive performance of the two generations of rats examined in this study. At the lowest dose level examined (0.5 ppm) there was a slight delay in the mean, age of vaginal opening and at 6 weeks of age effects were seen on the weight of the epididymides and/or seminal vesicles/prostate. Effects appeared more marked in the F2 pups than had been observed in the F1 pups of a comparable age. However, in terms of overall reproductive performance, trenbolone acetate exerted no effect at 0.5 ppm. The reproductive performance of all groups of F1 animals following withdrawal from treatment showed no marked difference from that of the control group.

Because the hormonal no effect level could not be determined in the proceeding multiple generation rat study, a supplementary study on the effect of trenbolone acetate on pregnancy of the rat and development of the offspring was conducted by Dr. Penny James at Huntington Research Centre, Huntington, England. At the initiation of this study trenbolone acetate was fed to 270 male and female rats at

levels of 0, 0.1, 0.3, 0.5, 3.0 and 18.0 ppm. The rats received the test diet at the above concentrations for 2 weeks prior to mating and throughout the mating period, gestation and lactation.

The adult males were sacrificed as the majority of litters approached weaning. Male pups were sacrificed on day 22 post-partum. All animals were subjected to post mortem examination. The following organs of all male pups were weighed prior to preservation: testes, seminal vesicles with prostate, and epididymides. On day 24 post partum the female pups and the parent females were sacrificed.

Treatment with trenbolone acetate at 18 ppm was associated with:

Slightly higher mean weekly body weight of F0 females but lower body weight gain during gestation and slightly lower weight gain of males over the last three weeks of treatment.

22/29 F0 females showed clitoral prominence at autopsy and all F1 female offspring were similarly affected from approximately 3 weeks of age.

A statistically significant extension and duration of gestation.

4/29 F0 females showed total litter loss.

Effects on litter parameters included reduced litter size, lower litter weight, marginally higher pup mortality and higher mean pup weight.

It is concluded that there is no deleterious effect of trenbolone acetate upon the weights of the testes, seminal vesicles with prostate or epididymides at the dosage level of 0.5 ppm. Therefore the hormonal no effect level for the rat is determined to be a dosage level of 0.5 ppm in the diet. The toxicological no effect level of trenbolone acetate as determined in these rat reproduction studies is not lower than that established as the hormonal no effect level in the Rhesus monkey.

SUMMARY

In the teratology study litter parameters as assessed by numbers of viable young, post implantation loss, litter and mean pup weight did not suggest any adverse effects of treatment. Embryonic and fetal development as assessed by the incidence of major malformations, minor visceral anomalies and skeletal variance was not adversely effected. The effect of trenbolone acetate on reproductive function of multiple generations in the rat showed the same hormonal effects as indicated under the toxicity studies summary of this same section. A conservative hormonal no-effect level for the rat was determined to be a dosage level of 0.5 ppm in the diet. This no-effect level is higher than that determined as the hormonal no-effect level in the Rhesus monkey. Therefore, the calculation of the safe concentration of total trenbolone acetate residues was based on the hormonal no-effect level as determined in the female monkey model system.

D. Pharmacology of Trenbolone Acetate

The effect of trenbolone acetate on protein synthesis in the rat was studied by B.G. Vernon and P.J. Buttery. These studies were published in the British Journal of

Nutrition , Vol. 36, pg. 575, 1976 and Vol. 40, pg. 563, 1978. The rats were injected with trenbolone acetate (800 µg/kg body weight) daily via the neck skin fold. The rats were sacrificed 7 and 14 days after initiation of treatment. Trenbolone acetate given subcutaneously to female rats increased their growth rate compared with that of the placebo treated controls. The increased growth rate of the trenbolone acetate treated rats was not a consequence of an increased water retention. The trenbolone acetate treated rats had significantly higher (P 0.01) total carcass nitrogen content but the total carcass fat content decreased by a nonsignificant 8.3%. There was evidence of a time lag in the response of the fractional synthetic rate of certain individual tissues to trenbolone acetate. The fractional synthetic rates of the uterus and skeletal muscle mixed tissue proteins were significantly reduced in the trenbolone acetate treated rats. The measured reduction in fractional synthetic rates was concluded to reflect true changes in the synthetic rate rather than a result of an alteration in the specific activity of the tyrosine pool used for protein synthesis.

An investigation of the androgenic and anabolic effects of trenbolone acetate was conducted by Hoechst AG, Frankfurt, W. Germany. This study was conducted by Dr. Schroder in castrated male rats. Seventy-five castrated male rats were given 10 daily doses of trenbolone acetate at 0, 0.02, 0.1 and 0.5 mg/per animal per day. The rats were treated subcutaneously. One day after the end of treatment the rats were killed and the musculus levator ani, the ventral prostate and the seminal vesicle were removed and the weight of these organs determined. The increase in the weight of the musculus levator ani is a measure of the anabolic effect of a substance and the increase in the weight of the ventral prostate and seminal vesicle a measure of its androgenic effect. In this study the androgenic and anabolic effects of trenbolone acetate were compared against a reference preparation of testosterone. The anabolic and androgenic effect of trenbolone acetate was about 5 times stronger than testosterone. At the same time, a small study was conducted to investigate the estrogenic effect of trenbolone acetate. The increase in weight of the uterus of castrated infantile rats was measured after treatment with estrogen active substances. Forty female rats were treated for four days with trenbolone acetate subcutaneously at daily levels of 0, 0.2, 1.0 and 5.0 mg/animal. On the fifth day the rats were killed and the weight of the uterus determined. These weights were compared with those of rats which served as controls or were treated with 17 beta estradiol. In this study trenbolone acetate demonstrated essentially no estrogenic activity being at least 1,000 times less active than 17 beta estradiol.

SUMMARY

The hormonal activity of trenbolone acetate can be divided into the anabolic antigonadotropic and androgenic effects. The anabolic effects of the compound relate to its ability to increase animal production through the improved efficiency of utilization of amino acids and subsequently increased protein accumulation inside muscle cells with the overall effect of improving average daily gain and improved feed efficiency in cattle. This anabolic effect has been well documented with slow release ear implants containing a total dose of 140 to 200 mg trenbolone acetate in cattle. This effective dose is gradually absorbed from the ear implant to give the desired effects on improvement in animal production. The antigonadotropic effect includes the inhibition of ovulation and testicular growth. A level of 3.0 ppm trenbolone acetate in the diet of rats will produce slight antigonadotropic effects (hormonal no-effect level in rats was determined to be 0.5 ppm in the diet). Levels of 960 micrograms trenbolone acetate per day in the diet of the Rhesus monkey will

produce an antigonadotropic effect. 240 microgram per day via the diet was established as the hormonal no-effect level in the Rhesus monkey. The androgenic activity of trenbolone acetate relates to its ability to stimulate the development of secondary sex characteristics of the male similar to the hormone testosterone. The androgenic effect on females would include coarse male-like hair, prominent pudendum and small ovaries. In the male the androgenic effect is measured by increases in the weight of the prostate and seminal vesicle. In cattle, females will demonstrate some androgenic effect of trenbolone acetate when an extremely high level of 3500 mg of trenbolone acetate is implanted. At the highest recommended dose of 200 mg trenbolone acetate no androgenic effects were seen.

E. Calculation of the safe concentration of total trenbolone acetate residues.

The results of the animal feeding studies indicated that the principle effects of trenbolone acetate were associated with the hormonal activity of the compound. The critical studies for assessment of human food safety therefore were related to the determination of the lowest level that would not produce hormonal effects in a female monkey model system. The hormonal no effect level was determined to be 40 µg/kg in the non human primate female.

From these data the safe concentration for residues was calculated to be 50 ppb in muscle, 100 ppb in liver, 300 ppb in kidney and 400 ppb in fat. The total average trenbolone acetate in beef liver was determined to be 43.8 ppb at 15 days after implantation and 50.5 ppb after 30 days. The residues in muscle, kidney and fat were much lower, see Section 6 F.

The safe concentration for residue of trenbolone acetate exceeds the total residues found in muscle, liver, kidney and fat at 15 and 30 days after implantation. It would not be expected that potential misuse of the product would result in tissue residue in excess of the safe concentration. Therefore, no withholding period prior to slaughter is indicated.

F. Residue depletion and metabolism studies.

The studies conducted on trenbolone acetate have resulted in determination of the total residues in the edible tissues of treated animals. These studies were conducted using tritiated trenbolone acetate. Studies of the biotransformation of the compound have been conducted with determination that in the liver of the bovine, there is production of 17 alpha hydroxytrenbolone whereas in the other tissues the primary metabolite is the 17 beta hydroxytrenbolone. The residues exist both free and in conjugated form.

The pivotal tissue residue study was conducted by Dr. D. R. Hawkins at Huntington Research Centre, Huntington, England. The purpose of the study was to determine the tissue residues of total radioactivity at 15 and 30 days after implantation with tritiated trenbolone acetate. 12 calves received subcutaneous implants in the ear containing 200 mg of tritiated trenbolone acetate. Six animals were sacrificed at each of 15 and 30 days after implantation. Blood samples were taken at intervals between dosing and sacrifice. At sacrifice the liver, kidneys and samples of muscle, fat and bile were taken for analysis. Concentrations of radioactivity and plasma were fairly constant during the experimental period, with mean levels of 4 to 5 ng/ml. Tissue concentrations of radioactivity were similar at 15 or 30 days. The highest

concentrations were found in the liver (means of 43.8 and 50.5 ng/g at 15 and 30 days respectively). Lower concentrations were found in the kidneys (16-22 ng/g) and muscle and fat (2 to 3 ng/g). High concentrations of radioactivity in bile (means of 1,163 and 741 ng/ml) indicate its importance in excretion of this compound. (See table 3).

Comparison of total and nonvolatile radioactivity concentrations showed that there was only a small amount of tritiated water produced. About 10% of the radioactivity in the liver samples was extracted by diethyl ether or ethyl acetate and this proportion increased to about 20 - 30% following incubations with betaglucuronidase, indicating the presence of a glucuronid(s) as a metabolite(s).

TABLE 3. TISSUE AND BILE CONCENTRATIONS OF RADIOACTIVITY IN CALVES SACRIFICED 15 AND 30 DAYS AFTER IMPLANTATION OF (3)H-TRENBOLONE ACETATE.

Results are expressed as ng equivalents trenbolone acetate /g or ml (ppb)

Tissue	15 day Mean	15 day S.D. *	30 day Mean	30 day S.D.
Liver	43.8	21.7	50.5	11.4
Kidneys	16.4	5.6	21.8	5.1
Muscle	2.41	0.65	3.23	0.50
Fat	2.45	1.15	2.40	0.88
Bile	1163	1046	741	148

* Standard Deviation

The metabolites of trenbolone acetate in the bile of the cow and the rat were studied by Dr. J. Pottier et al at the Research Center of Roussel Uclaf, Romanville, France and the Institute for Research in Animal Diseases, Berkshire, England. The bile transformations of trenbolone acetate, 17b-acetoxy-estra-4,9,11-triene-3-one (T.B.A.), in the bovine may differ from those in the rat which is the species used to determine its toxicity. Therefore, for reasons of public health, the metabolism in these animals species was compared. For this purpose, tritiated trenbolone acetate was injected intravenously to a heifer or to rats after catheterization of the common bile duct and the structures of metabolites were identified in the bile which is the major route of excretion in both species. For this purpose, 6,7(3)-H-T.B.A. was i.v. injected to a heifer or to rats and bile was collected for 24 hr. In both species the bile was by far the major route of excretion. The 3-keto trienic compounds accounted for the main part of extractable radioactivity before and after hydrolysis, showing the strong biological stability of this structure. In both species T.B.A. undergoes an extensive hydrolysis to 17beta hydroxy-estra-4, 9,11-triene-3-one (T.B.OH) and unchanged compound was not detected, but subsequent major metabolic pathways are different. In the rat, oxidation of the 17b-hydroxyl to estra-4, 9, 11-triene-3, 17-dione (T.B.O.) and hydroxylation in 16a-position are the major routes. The three main metabolites are T.B.OH and the 16a-hydroxylated derivatives of T.B.OH and T.B.O. In the heifer, 17a-epimerization is the major pathway and the main metabolite is by far the 17a-hydroxy-4,9,11-triene-3-one (Epi-T.B.OH). In both species, the other metabolites, resulting either from hydroxylation in 1, 2, 6a or 16b-

positions, or from aromatization of the A ring, were minor products. Thus, in the bovine species, the major pathway is similar to those of testosterone or 17b-estradiol which are mainly excreted as their epimers. This epimerization strongly decreases the biological potency of T.B.OH, as in the case of natural 17b-hormones, and leads to a detoxification of the possible residues in tissues used for human consumption. See Table 4 showing the relative amounts of the T.B.A. metabolites excreted in rats and cattle.

TABLE 4. QUANTITIES of 3-KETOTRIENIC COMPOUNDS EXCRETED IN THE RAT OR THE HEIFER BILE(a)
(Results are expressed in % of excreted radioactivity)

	Compounds	Rat	Heifer
I	T.B.A.(b)	-	-
II	T.B.OH(c)	20.6	.9
III	2-OH-T.B.OH	.6	-
IV	16a-OH-T.B.OH	10.5	.7
V	16b-OH-T.B.OH	3.4	-
VI	T.B.O.(d)	2.4	.9
VII	16a-OH-T.B.O.	17.1	1.3
VIII	16b-OH-T.B.O.	1.5	-
IX	1-OH-T.B.O.	1.8	.2
X	epi-T.B.OH(e)	-	34.7
XI	1-OH-epi-T.B.OH	-	.2
XII	16a-OH-epi-T.B.OH	-	3.0
XIII	16b-OH-epi-T.B.OH	-	3.0
XIV	6b-OH-epi-T.B.OH	-	.4

(a) The biles were collected during 24 hours after an i.v. injection of tritiated Trenbolone acetate.

(b) T.B.A. is the 17b-acetoxy-estra-4, 9, 11-trien-3-one.

(c) T.B.OH is the 17b-hydroxy-estra-4, 9, 11-trien-3-one.

(d) T.B.O. is the Estra-4, 9, 11-trien-3, 17-dione.

(e) Epi-T.B.OH is the 17a-hydroxy-estra-4,9,11-trien-3-one.

G. Tolerance for marker residue.

The study of the total tissue residues 15 and 30 days after administration of tritiated trenbolone, was conducted as discussed under F. above. The results of the 15 day total residues were such that they did not exceed the previous estimates of residues at 60 days. The highest concentration of radioactivity was found in the liver with a mean of 48 ppb. Lower concentrations were found in the kidney, muscle and fat. With the total residues at 15 days post implantation being of adequate safety margin when compared to the most sensitive toxicological or hormonal effects and the established principle that it is not expected that any animals will be intentionally slaughtered within 15 days after implantation, a zero withdrawal period was established. Therefore, identification of a marker residue was not attempted.

H. Withdrawal Period.

As discussed above no withdrawal period is required following the use of trenbolone acetate.

I. Regulatory Method

As discussed above no withdrawal time is required. Therefore, it is not necessary to have a Regulatory Assay Method or a confirmatory assay method for trenbolone acetate tissue residues.

V. AGENCY CONCLUSIONS

The Center for Veterinary Medicine has concluded that the data submitted in support of this new animal drug application comply with the requirements of Section 512 of the act. The data demonstrates that Finaplix® -H is safe and effective to increase the rate weight gain and improve feed efficiency in growing- finishing feedlot heifers. Finaplix® -S is safe and effective to improve feed efficiency in growing- finishing feedlot steers.

The Center has also concluded that Finaplix® -H and Finaplix® -S are safe for over-the-counter (OTC) distribution. Directions on labeling and packaging are adequate and ear implantation is a common method of administration of this type product within the feedlot industry. Producers who use this product can be expected to safely and successfully accomplish implantation. Further, there is no special need to recognize a disease condition, the drug is not a "controlled substance," and after implantation there is no need for medical monitoring or evaluation of the treated animal. Accordingly, prescription restriction of this product is not warranted.

VI. LABELING (Attached)

1. Finaplix® -H and Finaplix® -S package inserts
2. Finaplix® -H and Finaplix® -S cartridge labels
3. Finaplix® -H and Finaplix® -S carton labels
4. Finaplix® -H and Finaplix® -S shipper labels

Copies of these labels may be obtained by writing to the: Food and Drug Administration
Freedom of Information Staff (HFI-35)
5600 Fishers Lane
Rockville, MD 20857

Or requests may be sent via fax to: (301) 443-1726. If there are problems sending a fax, call (301) 443-2414.

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