

ENVIRONMENTAL ASSESSMENT REPORT

Finaplix<sup>R</sup> (trenbolone acetate)

Format following  
21 CFR 25.1 (j)

A. Date: April, 1987

B. Name of applicant: Sponsor Agent

Roussel-Uclaf  
Division Agro-Veterinaire Hoechst-Roussel Agri-Vet Co.  
163 Avenue Gambetta Route 202/206 North  
75020, Paris, France Somerville, N.J. 08876

In the United States Hoechst-Roussel Agri-Vet will be the distributor of the product.

C. Address: Hoechst-Roussel Agri-Vet Company  
Route 202/206 North  
Somerville, N.J. 08876

D. Environmental Information:

1. Describe the proposed action:

a. Purpose of the action:

A new animal drug application has been approved by the Food & Drug Administration for the use of a cattle ear implant, Finaplix<sup>R</sup>, which contains the active ingredient, trenbolone acetate. The ear implant improves average daily gain and/or efficiency of feed conversion in feedlot cattle (steers and heifers). The drug product is an ear implant for use in cattle at a dosage of 200 mg per animal for heifers and 140 mg per animal for steers. The ear implant will be manufactured by Roussel-Uclaf, Compiègne, France and shipped to the United States for distribution by Hoechst-Roussel Agri-Vet Co., Somerville, N.J. The use of this product is limited to growing, finishing feedlot cattle that are being grown for slaughter. Trenbolone acetate will be used as a partial replacement for existing agents intended for the same purpose. It is the feedlot industry practice to implant cattle when they arrive at the feedlot and to reimplant midway through the feeding period.

The cartridge containing the ear implants is designed to be used with a special implant gun. Each cartridge contains ten (10) ear implants. Each implant contains a number of pellets which make up the dose of trenbolone acetate. Each pellet contains 20 mg. trenbolone acetate.

There are ten (10) pellets in each implant for heifers and seven (7) pellets in each implant for steers.

b. The environment to be effected by the proposed action:

This approval allows the use of the active drug as produced in the Roussel-UCLAF facility, 63480 Vertolaye, France, and the formulated product as produced at USIPHAR, a subsidiary of Roussel UCLAF, Route de Choisy-au-Bac, 60205, Compiègne, France. The finished product will be imported, distributed and sold "over the counter" in the United States. The packages as prepared in France are not intended to be opened until immediately before use at the farm or feedlot. The cartridges contain a pre-measured dosage of the drug, avoiding any direct handling or spillage of the drug product. Unused dosages can be retained in the original cartridge until used.

The environments effected by the proposed actions are:

- o The manufacturing facilities in France.
- o The feedlots containing implanted cattle.
- o The agricultural lands receiving waste from treated animals.

2. Probable impact on the environment:

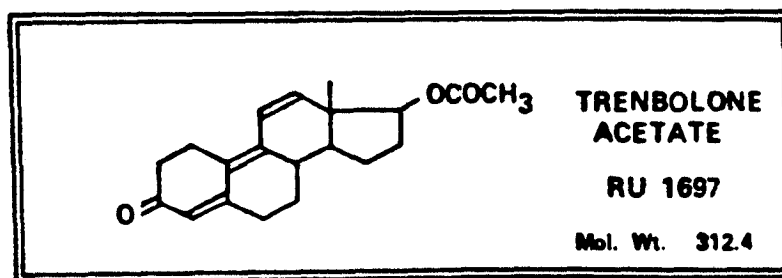
The probable adverse effects and beneficial environmental effects are described in the following sections.

CHEMICAL/PHYSICAL PROPERTIES

Finaplix<sup>R</sup> is a formulation of trenbolone acetate in a dry compacted pellet. Trenbolone acetate is the common name of the chemical:

17 b - (acetyloxy) estra-4,9, 11-trien-3-one

It has the Chemical Abstract Service Registry Number 10161-34-9. The following is the structural formula:



Molecular Formula		C <sub>20</sub> H <sub>28</sub> O <sub>3</sub>
Molecular Weight		312.39
Code Designation		RU 1697
WHO Number	2	2916

The principal physical and chemical properties are as follows:

- o Pale yellow, crystalline powder
- o Melting point: 95 - 97° C
- o  $n_D^{20}$ : + 39° to + 43° C (C = 0.5% methanol)
- o Dessication loss 1 g/100 g
- o E 1% / 1 cm: 920-980 (at 337  $\mu$  in ethanol)
- o Sulfuric ash 0.2 g/100g
- o Vapor pressure: 25° C
  - ps (trenbolone Acetate) =  $10^{-9}$ TORR
  - ps (TBOH - 17-alpha) =  $7 \cdot 10^{-10}$ TORR
  - ps (TBOH - 17-beta) =  $8 \cdot 10^{-11}$ TORR
- o Water solubility:
  - Trenbolone acetate - 17-21 mg/l
  - 17-alpha Trenbolone - 40-42 mg/l
  - 17-beta Trenbolone - 340-380 mg/l

### TOXICOLOGICAL/PHARMACOLOGICAL PROPERTIES

#### Toxicity Studies

The following toxicity studies were conducted to determine the toxicity of trenbolone acetate when given at various levels in the diet to rats, mice, pigs and Rhesus monkeys.

- o Rat oral toxicity study to determine reproductive effects on the F0 and F1 generations.
- o Long term chronic feeding study in mice to determine the potential tumorigenicity of trenbolone acetate on mice.
- o A long term chronic feeding study in rats following in utero exposure to determine the toxicity and tumorigenicity in rats.
- o A study to determine the hormonal no effect dose level for trenbolone acetate in the female Rhesus monkey.
- o A fourteen week feeding study to determine the oral toxicity of trenbolone acetate to growing pigs.

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:

- o Impairment of reproductive performance in rats.

- o Female rats had coarse male-like fur, perineal hair loss, prominent pudendum, small ovaries and cervix not palpable.
- o Male rats receiving high doses had an increase of small adrenals, small pituitaries, and lower prostate, testes, and kidney weights.
- o In the Rhesus monkey studies trenbolone acetate appeared to inhibit gonadotropin secretion and ovarian function.
- o 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 no-effect level was established at 40 microgram per kg per day (240 mg per day via the diet). Complete summaries of these studies can be found in the FOI summary of this NADA.

#### Mutagenic Potential Study Reports.

A complete battery of mutagenic potential studies were conducted with trenbolone acetate and its major metabolites 17 beta hydroxytrenbolone and 17 alpha hydroxytrenbolone. The following studies were conducted:

- o Ames metabolic activation test.
- o Mutagenic potential was assessed by examination of chromosome damage in somatic and germinal metaphase cells after oral administration to rats.
- o The mutagenic potential was determined by determining the effect of the two metabolites on cultured human lymphocytes.
- o The mutagenic potential was determined in an in vitro mammalian cell mutation assay.
- o A study was conducted to determine the Covalent Binding Index (CBI).
- o The mouse lymphoma L5178Y cell mutation test was conducted.
- o The Chinese hamster ovary/HPRT Locus Assay was conducted.
- o The SOS - Chromotest, the rec-assay and the V79 sister chromatid exchange test were all conducted to determine the mutagenicity of trenbolone acetate.

The results of these studies indicated that the trenbolone acetate, 17 alpha hydroxytrenbolone and 17 beta hydroxytrenbolone showed no significant evidence of mutagenic potential. Complete summaries of these reports can be found in the FOI summary of this NADA.

## Reproduction Study Reports

Several reproduction studies were conducted with trenbolone acetate because of its hormonal activity and to determine the teratogenic potential of trenbolone acetate. The following studies were conducted:

- o A teratology study in the pregnant rat.
- o The effect of trenbolone acetate on reproductive function of multiple generations in the rat.

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 (complete summaries of these studies and the calculations to determine the safe concentration of trenbolone acetate residues can be found in the FOI summary of this NADA).

## Pharmacology of Trenbolone Acetate

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.

Any human risk from consuming trenbolone acetate residues in meat can be calculated. In humans testosterone is active in daily doses of about 10 to 20 mg. given subcutaneously. With the assumption that trenbolone acetate has three times the activity of testosterone in humans, effects in man would be calculated to be in the range of a daily intake of 3 to 5 mg. trenbolone acetate (given subcutaneously). Trenbolone acetate demonstrates little if any hormonal activity when taken orally probably because of metabolism and poor absorption from the digestive tract. Therefore, trenbolone residues in meat from treated animals will be similarly ineffective in demonstrating any hormonal activity, thus insuring the human safety of this compound. The highest total residues of trenbolone acetate and its metabolites are found in the liver, all other edible tissues have much lower total residues. Total residue levels in liver correspond to approximately 50 ppb. This amount in the liver is equivalent to 0.05 mg. trenbolone per kg. of liver. A human would have to consume 60 kg. of beef liver in one day in order to consume the equivalent of 3 mg. of trenbolone acetate (the minimal effective dose given subcutaneously). This summary is taken from data presented in section 8 of the NADA and the studies presented in the FOI summary.

## METABOLISM BY TARGET ANIMALS

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 the following table). 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 beta-glucuronidase, indicating the presence of a glucuronid(s) as a metabolite (s).

TISSUE AND BILE CONCENTRATIONS OF RADIOACTIVITY IN CALVES  
SACRIFICED 15 AND 30 DAYS AFTER IMPLANTATION OF <sup>3</sup>H-TRENBOLONE  
ACETATE.

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

<u>Tissue</u>	<u>15 day</u>		<u>30 day</u>	
	<u>Mean</u>	<u>S.D.*</u>	<u>Mean</u>	<u>S.D.</u>
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

A non pivotal study was conducted at Huntington Research Centre by Dr. D. R. Hawkins to determine the bioavailability of trenbolone acetate residues in the edible tissues of heifers after oral administration to rats. Two heifers were implanted with pellets containing 300 mg tritiated trenbolone acetate. The two heifers were sacrificed at 60 days after implantation. The highest levels of radioactivity were present in the liver representing 28 and 31 ng trenbolone acetate per gram in the two heifers. The levels of radioactivity present in the kidneys was 19 and 29 ng/g and the concentrations of radioactivity in the fat were 1.0 and 1.5 ug/g. The radioactivity in the muscle (mean of 2.8 and 3.5 ug/g) samples were lower than those in the plasma (7.0 and 7.7 ug/g). After feeding the liver to rats, a mean of 3% and 81.3% of the radioactive dose was excreted in urine and feces respectively during 3 days. Similarly, after feeding ethyl acetate extracted liver, means of 4.8% and 77.8% were excreted in the urine and feces respectively during 3 days. After feeding the liver to bile duct cannulated rats means of 7.0%, 5.4% and 59.4% of the radioactive dose were excreted in the bile, urine, and feces respectively during 48 hours. The calculated mean absorption of the radioactive liver residue was 12.4%. After feeding the kidney to rats, a mean of 1.6% and 92.7% of the radioactivity was excreted in the urine and feces respectively during 3 days. Similarly, after feeding ethyl acetate extracted kidney means of 1.6% and 103.4% were excreted in the urine and feces respectively. After feeding the kidney to bile duct cannulated rats, means of 2.9%, 1.3% and 30.9% of the radioactive dose were excreted in the bile, urine and feces respectively up to 48 hours. The calculated mean absorption of the kidney radioactive tissue residue was 4.2%. After feeding freeze-dried muscle to rats, a mean of 6.5% and 84.9% of the radioactive dose was excreted in urine and feces respectively during three days. Similarly, after feeding ethyl acetate extracted muscle means of 2.1% and 72.8% were excreted in urine and feces



respectively. After feeding the muscle to bile duct cannulated rats, means of 2.7%, 2.3% and 56% of the radioactive dose were excreted in bile, urine and feces respectively during 48 hours. The calculated mean absorption of the muscle radioactive tissue residue was 5.0%.

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, Romainville, 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<sup>3</sup>-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 17b 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 a-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 the following table showing the relative amounts of the T.B.A. metabolites excreted in rats and cattle.

QUANTITIES of 3-KETOTRIENIC COMPOUNDS EXCRETED IN THE RAT  
OR THE HEIFER BILE<sup>a</sup>

(Results are expressed in % of excreted radioactivity)

	<u>Compounds</u>	<u>Rat</u>	<u>Heifer</u>
I	T.B.A. <sup>b</sup>	-	-
II	T.B.OH <sup>c</sup>	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. <sup>d</sup>	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 <sup>e</sup>	-	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.

## ENVIRONMENTAL FATE

Studies reporting the metabolism and migration of the waste products of the drug following feeding to cattle have been conducted and are summarized in this section. These reports include the characteristics of radio labelled trenbolone metabolites with respect to soil migration, biodegradation in soils and related studies.

The potential for trenbolone and its metabolites to bioaccumulate in organisms in the environment was tested by determining the n-Octanol/water partition coefficient (See attachments 1 and 2). The partition coefficient is expressed as:

$$P = \frac{C(n - Octanol)}{C(water)}$$

P = Partition Coefficient

C = Trenbolone concentration

The n-Octanol/water partition coefficients for trenbolone acetate and its metabolites were found to be:

o trenbolone acetate	P = 5898
o 17 beta trenbolone	P = 1040
o 17 alpha trenbolone	P = 510

Based on these partition coefficients trenbolone acetate and its metabolites would be expected to have a low potential for bioaccumulation in organisms in the environment. (Reference: New and Revised Chemical Fate Test Guidelines, October 1984; U.S. Environmental Protection Agency, Washington, D.C. pages 1 - 26).

A kinetic study on the degradation of trenbolone acetate (RU-1697) at 4 temperature levels was conducted by Roussel-Uclaf, Paris, France. The study was conducted over a period of 3 years (36 months) and the stability was tested at temperatures of 0°, 20°, 37°, and 50°C. Trenbolone acetate was stable when held at 0°C. for the three year period. Trenbolone acetate was not stable at the higher temperatures and the degradation increased as the temperature increased (see attachment 3).

A study was conducted by Dr. A. Grandadam in the Department of Research and Development, Roussel Uclaf, Paris, France to verify the destruction of trenbolone acetate and 17 beta trenbolone during cooking of meats into which it has been incorporated. Chopped meat was impregnated with an oil solution, trenbolone acetate or 17 beta trenbolone at levels of 3 mg per 100 gms. of meat. The chopped meat was then subjected to the following four treatments:

- o Rare steak: 30 seconds of cooking on each side.
- o Medium rare steak: 1 minute of cooking on each side.
- o Well done steak: 2 minutes on each side.
- o Long term cooking: Chopped meat was placed in sealed plastic bag and cooked in boiling water for one hour.

Trenbolone acetate and 17 beta trenbolone are destroyed by cooking. Their loss is proportional to the cooking time. During cooking the trenbolone acetate did not

metabolize into 17 beta or 17 alpha trenbolone. Degradation of trenbolone acetate and 17 beta trenbolone varied from 7 to 80% depending upon cooking time (see attachment 4).

The vapor pressure of trenbolone acetate, 17 alpha hydroxytrenbolone and 17 beta hydroxytrenbolone was determined in a study conducted by Roussel Uclaf of Paris, France (see attachment 5). The gas saturation method was used for this study as it is suited to the measurement of vapor pressure of organic compounds of low volatility. The vapor pressure of these three compounds at 25°C. was evaluated as:

- o Trenbolone acetate =  $10^{-9}$ TORR
- o 17 alpha hydroxytrenbolone =  $4 \cdot 10^{-10}$ TORR
- o 17 beta hydroxytrenbolone =  $8 \cdot 10^{-11}$ TORR

A stability study on the metabolites of trenbolone acetate was conducted by Roussel Uclaf of Paris, France. 17 alpha trenbolone and 17 beta trenbolone were placed in solution and subjected to the following conditions:

- o held at 50°C and protected from light for 24 and 96 hours.
- o exposed to a sun-test\* for 6 hours at room temperature.
- o exposed to natural light\*\* for 12 and 48 hours.
- o exposure to UV (366 nm) for 4 hours.
- o exposure to UV (366 nm) for 6 hours.

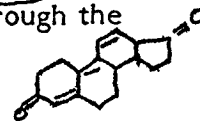
\*A 90-klux light with a spectrum similar to white light (the main radiation coming from the sun).

\*\*Also referred to as daylight. The material kept in these conditions was placed in a room receiving daylight but was not exposed directly to sunlight.

The two metabolites were stable when exposed to temperatures of 50°C in the absence of light. The two metabolites degraded over time with exposure to light. The 17 beta metabolite was more sensitive to exposure to light than the 17 alpha metabolite (see attachment 6).

A study on the biodegradation in soils of 17 alpha and 17 beta trenbolone was conducted at Huntington Research Centre by Dr. J. N. Hossack. The purpose of the study was to determine the biodegradability in soils of 17 alpha and 17 beta trenbolone, the two major metabolites of trenbolone acetate. The study was conducted following the principles and procedures of section 10:12 of the Environmental Technical Handbook of the Center for Veterinary Medicine, Environmental Impact staff. In the sandy loam and clay loam soil the test compounds showed an initial small amount of biodegradation (up to 3%) fairly rapidly and thereafter reached a plateau of about 5%. In the silt loam soil, a slow rise in biodegradation was seen. But at termination of the experiment after 56 days incubation, degradation as measured by CO<sup>2</sup> production had reached only 11% and 21% for the 17 alpha and 17 beta trenbolone respectively. It was clear that at the arbitrary concentrations used in this study, the test compounds did not appear to be readily biodegradable. In two of the three soils they were inhibitory to microbial metabolism as indicated by CO<sup>2</sup> production. It was concluded that more testing was needed using concentrations more closely approximating the levels likely to be found in soil. Following the completion of this study at Huntington Research Centre the soils were sent to Roussel Uclaf, Paris, France where they were analyzed by reversed-phase HPLC and ultraviolet detection for the residual amounts of 17 alpha and 17 beta

trenbolone. Only very low concentrations of the compounds were recovered corresponding to 0.25 to 2% of the 17 alpha trenbolone and 2 to 5% of the 17 beta trenbolone originally added to the soils at a concentration of 250 ug/g. The loss of the compounds is presumed to be due to biodegradation. Evidence of biodegradation was substantiated by recovery from the soils of a metabolite identified as a 17 keto-trienic compound having an HPLC retention time corresponding to that of triendione. Thus degradation of the two metabolites occurred even though measurement through the classical evolution of CO<sub>2</sub> could not be demonstrated (see attachment 8).



A soil Adsorption/Desorption study was conducted with tritiated 17 alpha hydroxytrenbolone. This study was conducted by Analytical Biochemistry Laboratories Inc., Columbia, MO. Aqueous solutions of this tritiated compound were equilibrated with 3 soil types and the adsorption coefficients and absorption constants were determined. Liquid scintillation counting analysis was employed to measure test material concentrations in the aqueous phase. The mobility of a chemical through soil can be directly related to its adsorption properties. K<sub>oc</sub> values can be used to rank and compare chemicals with respect to their leaching potential. See the following table for the results from this study.

<u>Soil Type</u>	<u>Stage of Study</u>	<u>K<sub>oc</sub></u>	<u>Mobility Class</u>
Sandy loam	Adsorption	477	Medium
	Desorption	1390	Low
Clay	Adsorption	1100	Low
	Desorption	2560	Slight
Loam	Adsorption	420	Medium
	Desorption	1550	Low

A soil Adsorption/Desorption study was conducted by Analytical Biochemistry Laboratories, Inc. Columbia, Mo. to determine the adsorption/desorption constants of tritiated 17 beta hydroxytrenbolone. Aqueous solutions of tritiated 17 beta hydroxytrenbolone were equilibrated with 3 soil types and the adsorption/desorption coefficients and adsorption/desorption constants were determined. Liquid scintillation counting analysis was employed to measure test material concentrations in the aqueous phase. The following table contains the results of this study:

<u>Soil Type</u>	<u>Stage of Study</u>	<u>K<sub>oc</sub></u>	<u>Mobility Class</u>
Sandy loam	Adsorption	1300	Low
	Desorption	4180	Slight
Clay	Adsorption	3060	Slight
	Desorption	9570	Immobile
Loam	Adsorption	1010	Low
	Desorption	3820	Slight

The results of these two adsorption/desorption studies indicate that the 17 beta metabolites mobility in soil is low to very low. The mobility of the 17 alpha metabolite is ranked as low to medium (see attachments 10 and 11).

### Estimated amounts of trenbolone and metabolites that will enter the environment.

The only biological activity of trenbolone acetate that has been identified is its hormonal activity. The "no-observed-effect level" was established at 40 mcg/kg. The major metabolite excreted by cattle is 17 alpha hydroxytrenbolone which is at least 25 times less active hormonally than trenbolone acetate (see toxicological/pharmacological properties section of FOI). The metabolism data (from sections 7 and 8 of the NADA) demonstrate that no parent trenbolone acetate is excreted by cattle and therefore no parent compound is expected to be found in the soil or runoff from feedlots. The major metabolite (17 alpha) represents approximately 34.7% of excreted radioactivity. Trenbolone metabolites other than 17 alpha are present in cattle excreta as less than 3% of measured radioactivity. Therefore based on the following calculations neither trenbolone or the majority of its metabolic products are expected to cause adverse impacts on environmental organisms. The estimation of the excretion of total residues and the major metabolite (17 alpha) in the excreta of treated animals can be calculated as follows:

This calculation assumes a feed lot of one acre in area, populated by 110 head of cattle which are implanted with trenbolone acetate, 200 mg, with depletion of the implant over 66 days. It is assumed that the excreta from the animals will be 5 liters of urine and 5 kg of feces per day for a total of 10 kg of waste per animal.

#### a. Concentration in excreta from implanted animals:

The excretion of the 200 mg trenbolone acetate in the implant over 66 days would give an average daily excretion of 3 mg of trenbolone metabolites per day. 3 mg would be diluted in 10 kg of waste or 0.3 mg/kg of animal waste. 0.3 mg/kg is equal to 0.3 ppm total residues. The amount of the 17 alpha metabolite in this total residue is approximately 0.1 ppm (34.7% of total residues).

#### Concentration in water run-off from feedlot:

##### Assumption:

Each animal will excrete all of the implanted trenbolone acetate (200 mg). During the feeding period there will be 2 inches of rain fall.

Two inches of rainfall is sufficient to wash away all of the drug and drug metabolites. There will be complete removal of drug into the runoff. This presents the worst case or maximum possible concentration in the run-off. It is theoretically possible to have all of the residues in the run-off because the solubility of the major metabolite is 40-42 mg/l. However, based on the soil Adsorption/Desorption study a portion of the residues would adhere to the manure and feedlot soil.

Two inches of rain will weigh 205,500 kg/acre.

Therefore,

- o The amount of trenbolone residues excreted equals:  
110 animals x 200 mg = 22,000 mg or 22 grams.
- o  $\frac{22,000 \text{ mg/acre}}{205,500 \text{ kg H}_2\text{O/acre}} = 0.107 \text{ mg/kg} = 0.107 \text{ ppm} = 107 \text{ ppb}$
- o Maximum concentration in run-off is 107 ppb. The maximum concentration of the major metabolite would be 37.1 ppb.
- b. Concentration of trenbolone acetate in soil due to fertilization with waste from implanted animals:

Assume the following circumstances:

- (1) Drug equivalents from the combined urine and feces will find their way into excreta to be spread on the land.
- (2) The incorporation rate of manure into soil is 15.0 metric tons per acre.
- (3) The excreta will be incorporated into the top 6" of soil of one acre of land weighing 909,000 kg.

Therefore:

- o Concentration of drug equivalents in excreta as determined above is 0.3 ppm.
- o In one metric ton:  
 $0.3 \text{ mg} \times 1000 = 300 \text{ mg}$  of drug equivalents
- o In one acre of spreading therefore there is:  
 $300 \text{ mg} \times 15.0 \text{ metric tons} = 4,500 \text{ mg/acre}$
- o  $\frac{4,500 \text{ mg/acre}}{909,000 \text{ kg}} = .005 \text{ mg/kg} = 5 \text{ ppb}$
- o  $5 \text{ ppb (total residues)} \times 34.7\% = 1.7 \text{ ppb}$  of the major metabolite (17 alpha)

As indicated by the above calculations, the amount of trenbolone metabolites that would be released into the soil and water as the result of the use of trenbolone acetate implants and subsequent disposal of the feedlot waste is extremely small. These calculations assume no decomposition of the drug and its metabolites upon being excreted.

Based on the soil degradation study a maximum of only 2% of the initial concentration of the major metabolite was found in soils after 56 days. Therefore, in less than 2 months after incorporation in the soil the remaining 17 alpha trenbolone would be 0.03 ppb or less.

Based on the degradation, solubility and the soil sorption coefficients of the 17 alpha metabolite, the maximum amount of 17 alpha that could be expected in agricultural soil runoff is approximately 10% of the amount in the soil. This 10% estimate is conservative and allows for a 20 times safety factor based on a paper titled, "The Pesticide Content of Surface Water Draining from Agricultural Fields - A Review" by R. D. Wauchope (J. Environ. Qual., Vol 7, No. 4, 1978, page 459). Therefore the maximum amount that could get into soil run-off could not exceed 0.17 ppb.

- c. Estimation of the concentration of trenbolone acetate waste from the slaughter house:

Assumptions,

- 1) There will be an average of 30 mg of trenbolone in each animal's ear
  - 2) 100 kg of offal and packing house removed bones/per animal
  - 3) All animals slaughtered will be implanted
- Therefore:  
 $30 \text{ mg}/100 \text{ kg} = .3 \text{ mg}/\text{kg} = .3 \text{ ppm}$

- d. Estimation of the concentration of trenbolone and break down products in rendered products:

Assumptions,

- 1) Dry matter content of incoming packing house waste is 50%
- 2) Tallow yield from incoming material is 16.6%
- 3) Meat and bone meal yield is 33.4%
- 4) All trenbolone is concentrated in tallow portion

Therefore:

$$0.3 \text{ ppm} / .166 = 1.8 \text{ ppm}$$

It is not expected that the trenbolone acetate will survive the rendering operation. Trenbolone acetate is not stable at high temperatures (See attachments 3 and 4).

With the evidence as stated above the environmental fate of trenbolone acetate and its metabolites can be summarized as follows:

- o The levels of trenbolone metabolites in soil following fertilization is conservatively estimated to be a very low 5.0 ppb.



- o The soil migration potential for the metabolites of trenbolone acetate is small.
- o Trenbolone acetate and its metabolites are bio-degradable in soil and water.
- o Runoff of the metabolites of trenbolone acetate into bodies of water is not likely because of the low concentrations in the soil and the binding properties of the metabolites to soil.

### 3. Solid and Liquid Waste

Care is taken to minimize the waste of intermediates and final product in the manufacture of trenbolone acetate. All wastes which are generated in the manufacture of trenbolone acetate are handled in compliance with the local and federal regulations in France (see attachment 7). Waste liquid effluence are transferred to a biological treatment station with recovery and/or incineration. The solid waste is picked up by specialized firm for further treatment or disposal at an authorized site. In the manufacture of the formulated product there is essentially no wastage of product and no impact on the environment. In France the plants manufacturing the active ingredient, trenbolone acetate, operate under the surveillance of a French government agency which is charged with enforcing the regulations enacted in the field of environmental protection.

### 4. Toxic substances

The use of this product will introduce no heavy metals, pesticides, radiation or other highly persistent or toxic materials into the environment. The toxicity of trenbolone acetate has been assessed in lifetime feeding studies, in reproduction studies and in a teratogenicity study. There has been no indication of insidious toxicity of the compound.

### 5. Populations

The only population which will be effected by this animal drug is the intended effects upon cattle receiving trenbolone acetate. The drug is not intended for direct use in humans and there is little liklihood that human population will be directly exposed. The product comes packaged in a cartridge with the chance of direct human exposure to the drug being minimal.

Target animal safety studies have been conducted with dosages as high as 3500 mg per calf given as a single implantation, or 1,000 mg per animal given on two occasions with an interval between implants of 63 days. There was no adverse effects on the health of the animals. Body weight gains were increased in comparison with the controls and the hematological and blood chemistry parameters generally remained within normal limits with any effects being consistent with the hormonal activity of the compound.

A study was conducted by Huntington Research Centre to determine the effects of 17 alpha and 17 beta trenbolone on soil carbon and nitrogen cycle microorganisms. The procedures used followed the principles of the OECD chemicals testing program "Guidelines for Assessing the Toxicity of Chemicals to Soil Microorganisms", 4th draft, 1981. In this procedure the evolution of carbon dioxide from soils containing the test compound is compared with control soils (carbon cycle) and ammonification and nitrification in soils containing the test compound or compared with control soils (nitrogen cycle). The maximum expected concentration of the major metabolite (17 alpha) in soils was calculated as being .0017 ppm. The levels tested in this study were control, 0.015ppm of each metabolite and 0.15 ppm of each metabolite (88 times the maximum calculated level of the major metabolite). For the carbon cycle, the test soils and control soils were placed in duplicate respirometers and the amount of carbon dioxide evolved was measured over a period of 63 days. For the nitrogen cycle, quadruplicate samples were set up for analysis at intervals up to 40 days of incubation. The soil samples were analyzed for their ammonium, nitrate, and nitrite levels. In the carbon cycle tests no significant evidence of inhibition of respiration was seen by either compound in the sandy soil, while in the clay soil all of the treatment groups showed a statistically significant stimulation of respiration compared to the control. In the nitrogen cycle tests there appeared to be a similar trend toward an increase in activity in the sandy soil but with only the higher treatment level of 17 alpha trenbolone giving a statistically significant increase in nitrate levels. In the clay soils after 42 days incubation there were no visual or statistically significant differences between the treated and control groups. It was concluded that the metabolites of trenbolone acetate, 17 alpha trenbolone and 17 beta trenbolone are not expected to have any biologically significant environmental effects on soil carbon and nitrogen cycling microorganisms or the respective soil processes (see attachment 9).

No parent trenbolone is expected to be present in the environment based on the metabolism data. Trenbolone metabolites other than 17 alpha are present in cattle excreta as less than 3% of measured radioactivity. The major metabolite, 17 alpha, represents approximately 34.7% of excreted radioactivity (see environmental fate section of EA). The highest level of 17 alpha trenbolone used in the soil carbon and nitrogen cycle studies is at least 30 times higher than the highest calculated amount (1.7 ppb) expected to get into the soil. Therefore, neither trenbolone or its metabolic products are expected to cause an adverse impact on environmental organisms.

The hormonal no-effect level in rats was determined to be 0.5 ppm in the diet and an intake of 0.24 ppm per day in the diet of the Rhesus monkey is the no-effect level for this species. The highest concentration of the major metabolite in the environment will be in the feedlot runoff and assuming a worst case that level is 0.037 ppm or 6.50 times less than the no-effect level in the diet of the Rhesus monkey. Additionally, the major metabolite (17 alpha) is 25 times less active hormonally than trenbolone acetate which increases even more the environmental safety factors (taken from toxicological/pharmacological properties and environmental fate sectors of this environmental assessment). Therefore, levels of 17 alpha trenbolone in the environment will not cause a significant impact in man or other animals in the environment.

Compounds similar to trenbolone acetate have not shown antimicrobial activity. Studies conducted for the NADA indicate that trenbolone acetate also is not harmful to microorganisms (see attachment 9 and the summaries of the mutagenicity studies in the FOI).

#### 6. Human Values

With the proper use of this animal drug there will be no adverse effects upon public health or the human environment. The drug is not intended for use in humans and is not habit forming.

#### 7. Food Contamination

This new animal drug application contains analytical methods for residues, including studies to determine the actual levels of residues in the edible products from cattle implanted with trenbolone acetate. It is concluded that the residues of trenbolone acetate are not of toxicological significance when used as labelled. The analytical method is adequately sensitive to measure residues of less than 30 ppb.

The metabolism of trenbolone acetate has been studied in the target animal. Trenbolone acetate is rapidly hydrolyzed to trenbolone and both trenbolone and its metabolites are rapidly excreted as glucuronides and sulfates, mostly in the bile. The predominant metabolites identified in the extractable fractions of bile in the rat are trenbolone and a 16-OH and a 17-keto metabolite; while in cattle it is mainly 17 alpha hydroxytrenbolone with small amounts of trenbolone and other metabolites. The parent compound and its metabolites have low oral activity.

Chronic feeding (carcinogenicity) studies have been conducted in both rats and mice. Both studies were lifetime studies and the rat study included an in utero exposure. The effects of trenbolone acetate were consistent with its hormonal activity with effects only upon endocrine target tissues in both rats and mice. A reproduction study in rats has been conducted which includes an assessment of potential teratogenic effects. No unusual or unexpected findings were made. Studies to determine the hormonal no effect level were conducted in the Rhesus monkey. Results of the studies in monkeys suggest that the primate is less sensitive to the compound than was observed in other species. The overall food safety margin for residues of trenbolone acetate in edible animal tissues is at least two-hundred fold.

8. Natural Resources

There is no significant depletion of natural resources associated with the approval of this new animal drug application. There is expected to be no effect on the depletion of natural resources due to the manufacture of the drug.

9. Energy

The indirect effect of approval of this NADA will be a saving of energy by the improved average daily gain and improved feed efficiency of feedlot cattle resulting in the more efficient use of feed resources and a shorter period of time in the feedlot. In the manufacture of the new animal drug, there is negligible demands for energy or use of petrochemicals.

E. Describe measures taken to avoid or mitigate potential adverse environmental effects:

In light of the data presented above, no such measures are necessary. No adverse effects of any significance have been determined.

F. Analyzed environmental impact of the manufacturing process of the article that

is the subject of the requested action:

An outline of the manufacturing process for trenbolone acetate is included in Section 5 of the NADA. The synthesis of this new animal drug substance is contained within the manufacturing facility. Trenbolone acetate will be manufactured and packaged into the final dosage form in France. The finished dosage form will then be shipped to the United States for distribution.

Occupational exposure is controlled during the production and packaging of trenbolone acetate by strict adherence to Good Manufacturing Practices. The various stages of the production of Finaplix<sup>®</sup> are housed in special rooms in which only Finaplix is manufactured. These areas are constructed so they are easily cleaned and maintained such as; smooth painted wall surfaces, glossy floors, smooth false ceilings with built-in lamps and jointing-free construction. All the manufacturing and packaging premises are designed to ensure a maximum degree of pharmaceutical safety. The specific manufacturing areas are divided into sections in which only certain manufacturing steps are performed in order to avoid any accidental cross-contamination. The manufacturing operations are done in completely closed rooms fed with filtered and climatized air heated at 19°C to 26°C (depending on the external temperature) and having a relative humidity of  $55 \pm 5\%$ . The air change rate ranges between 12 and 48 times per hour depending upon the specific operation being performed. A slight underpressure is maintained between the various rooms to avoid any possible cross-contamination. Personnel are protected against accidental exposure to trenbolone acetate by wearing required garments which cover the entire body including eye protection and nose masks (see attachment 7 and taken from manufacturing section of NADA).

The sponsor for this new animal drug application certifies that any emissions resulting from the manufacturer of trenbolone acetate will be in full compliance with the appropriate regulations of the country of manufacture (see attachment 7).

#### G. Specific Data

In the appendix to this Environmental Assessment are reports of studies intended to elucidate the effects of trenbolone acetate on the environment. The attached reports are as follows:

<u>Report</u>	<u>Page #</u>
<u>Attachment 1:</u> n-Octanol/water partition coefficient of trenbolone acetate. Study #STE-117-A-02-1, October, 1984.	
<u>Attachment 2:</u> n-Octanol/water partition coefficient of 17 alpha and 17 beta trenbolone. Study #STE-117-A-04, December, 1984	

<u>Report</u>	<u>Page #</u>
<u>Attachment 3:</u>	
Kinetic study on degradation of trenbolone acetate (RU 1697) at 4 temperature levels. ...	70
<u>Attachment 4</u>	
The effect of cooking on the stability of trenbolone acetate and 17 beta trenbolone, Roussel-Uclaf, Paris, France, Department of Research and Development, Dr. A. Grandadam, March 31, 1984. ...	71
<u>Attachment 5</u>	
Determination of vapor pressure of trenbolone acetate, 17 alpha hydroxytrenbolone and 17 beta hydroxytrenbolone. Study #TE-117-A-05-1, January, 1985. ...	75
<u>Attachment 6:</u>	
Stability study on the metabolites of trenbolone acetate: 17 alpha trenbolone and 17 beta trenbolone. Study #STE-117-A-09-1. September, 1985. ...	79
<u>Attachment 7:</u>	
Statements of Compliance from Roussel Uclaf, Paris, France with the Environmental and Occupational laws of France. ...	83
<u>Attachment 8:</u>	
Biodegradation in soils of 17 alpha and 17 beta trenbolone, Huntington Research Centre, Huntington, England, July 1986. Study #RSL707/86437. ...	87
<u>Attachment 9:</u>	
The Effects of 17 alpha and 17 beta trenbolone on soil carbon and nitrogen cycle microorganisms, Huntington Research Centre, Huntington, England, September 1986. Study #RSL724/861116. ...	94
<u>Attachment 10:</u>	
Determination of Adsorption/Desorption constants of tritiated trenbolone 17 alpha OH Analytical Biochemistry Laboratories, Inc. Columbia, MO, July 30, 1986. Report #34521. ...	110
<u>Attachment 11:</u>	
Determination of Adsorption/Desorption constants of tritiated trenbolone 17 beta OH, Analytical Biochemistry, Laboratories, Inc., Columbia, MO. July 30, 1986, Report #34589. ...	145

- H. Describe the probable adverse environmental effects that cannot be avoided

There are no known adverse environmental effects related to the manufacture and use of this new drug.

- I. Evaluate alternatives to the proposed action

The only alternative to approval of the new animal drug application is non approval. This would mean that the cattle industry would not have the choice of use of this product. Without the availability of growth promoting products, the adverse effects on the cattle industry would be increased cost of production and increased depletion of feed supplies through less efficient production.

- J. Describe the relationship between local and short term use of the environment with respect to the proposed action and the maintenance and enhancement of long term productivity.

This action will not require any significant use of the environment. There is no expectations or evidence to expect short term or long term effects.

- K. Describe any irreversible and irretrievable commitments of resources that would be involved if the proposed action should be implemented.

None.

- L. Discuss the objections raised by other agencies, organizations or individuals that are known by the applicant.

No objections have been raised by an agency, organization or individual. The drug has a history of safe manufacture and use in France. It has been authorized by that government for manufacture and it has been authorized for use in cattle in other countries.

- M. If the proposed action should be taken prior to 90 days from this circulation of a draft environmental impact statement or thirty days from the filing of a final environmental impact statement, explain why.

It is the sponsor's position that this action will not require the preparation of an Environmental Impact Statement. If it is determined that environmental impact statement is required, the petitioner has no objection to the timing as presented above.

N. Risk - Benefit analysis


It is the sponsors belief that there is no significant risk associated with the proposed action. We have demonstrated in the application the usefulness of trenbolone acetate for feedlot cattle. The significant benefits of approval of this NADA far outweigh any potential risks.

O. Certification

The undersigned petitioner certifies the information furnished in this environmental assessment report to be true, accurate and complete to the best of his knowledge.

Date

4/8/87

  
Manager, Nutritional Research

4/87