Synovex® Plus (trenbolone acetate and estradiol benzoate) Implant

Environmental Assessment

1. DATE

November 1995

2/3. NAME AND ADDRESS OF APPLICANT

Syntex Animal Health
Division of Syntex Agribusiness, Inc.
3401 Hillview Avenue
Palo Alto, CA 94304

4. DESCRIPTION OF PROPOSED ACTION:

A New Animal Drug Application (NADA 141-043) has been submitted in support of the use of Synovex® Plus (trenbolone acetate and estradiol benzoate) Implants in steers fed in confinement for slaughter. Synovex® Plus Implants contain 200 mg of trenbolone acetate and 28 mg of estradiol benzoate in an eight pellet dose. The implant dose (eight pellets) is administered by subcutaneous implantation in the middle one third of the ear. Synovex® Plus Implants improve feed efficiency in steers fed in confinement for slaughter.

Essentially all feedlot steers are currently implanted with growth-promoting hormones at the beginning of the feeding period. Approximately 13 million steers per year are treated with implants containing trenbolone acetate (140 mg of trenbolone acetate alone or 120 mg trenbolone acetate in combination with 24 mg estradiol). Approval of the Synovex® Plus Implant product will not significantly increase the overall use of growth-promoting hormone implants, specifically those containing an anabolic steroid component such as trenbolone acetate and/or an estrogenic hormone component such as estradiol or estradiol benzoate. Synovex® Plus Implants will compete with other similar products in the marketplace and will routinely be used in place of other such implant products.

Approval of this NADA will authorize the production and use of drug substances and formulated product as indicated below:

Synthesis of the drug substances will occur at the following sites:

1) Syntex Chemicals, Inc.
   2075 North 55th Street
   Boulder, CO 80301

2) Syntex S.A. de C.V. - Chemicals Division
   Km.4 Carretera Federal
   Cuernavaca - Cuautla
   62500 Jiutepec, Morelos, Mexico

Manufacture of the drug product will occur at the following site:

Syntex Laboratories, Inc.
3401 Hillview Avenue
Palo Alto, CA 94304

This drug product will be used in feedlots throughout the United States, therefore use will not be concentrated in any specific geographical location.

Returned drug product in the United States will be sent to a Fort Dodge Laboratories facility located in either Fort Dodge, Iowa or Wilmington, Ohio, where it will be packaged and shipped for incineration as nonhazardous material at an approved incinerator.

The types of environments present and adjacent to the production facilities for the drug substances and drug product are described below:

Syntex Chemicals, Inc. - Boulder, CO
This facility is located in the foothills of the Rocky Mountains. The immediate area is primarily undeveloped with a few adjacent industrial facilities. The climate is temperate.

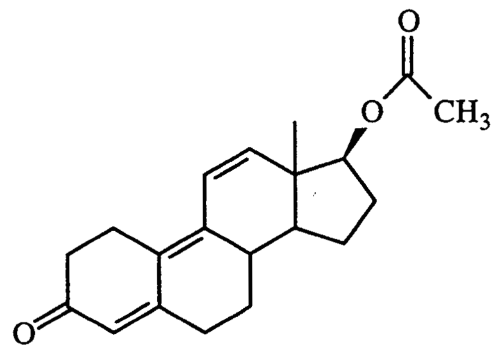
Syntex S.A. de C.V. - Cuernavaca, Mexico
This facility is located in the CIVAC industrial park within the Cuernavaca Valley. The climate is tropical and subhumid. To the north are the Sierra Chichinautzin Mountains.

Syntex Laboratories, Inc. - Palo Alto, CA
This facility is located in the San Francisco Bay area, in the suburban/light industrial area of Palo Alto. The climate is temperate.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

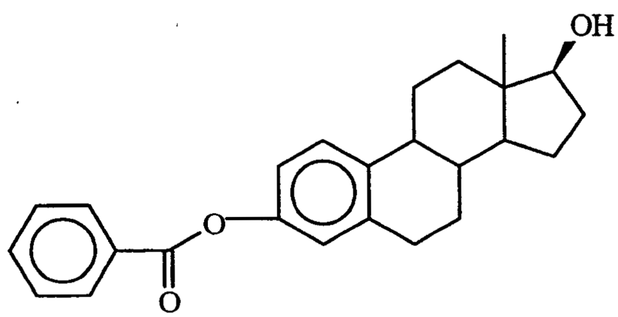
Trenbolone Acetate (TBA)

The chemical name for trenbolone acetate is 17β-acetyloxyestra-4,9,11-triene-3-one and its CAS number is 10161-34-9. Its empirical formula is $C_{20}H_{24}O_3$ and its formula weight is 312.41. Trenbolone acetate is a creamy white crystalline powder with a melting point of 96-97°C (Merck Index). Its structural formula is presented below.



Estradiol Benzoate (EB)

The chemical name for estradiol benzoate is estra-(1,3,5(10)-triene-3,17-diol,(17β)-3-benzoate and its CAS number is 50-50-0. Its empirical formula is $C_{25}H_{28}O_3$ and its formula weight is 376.49. Estradiol benzoate is a white crystalline powder with a melting point of 191-196°C (Merck Index). Its structural formula is presented below.



6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

The identities of components, reagents, and solvents used in the manufacture of trenbolone acetate and estradiol benzoate are described elsewhere in NADA 141-043. Whenever possible, the compounds used and/or produced in the manufacture of the drug substances are recycled back into the process or, when recycling is infeasible, disposed of in accordance with appropriate laws and regulations. Each facility has a spill prevention and control plan which specifies the handling of an accidental release of any hazardous material into the environment.

Manufacturing controls and permit information are described below for each facility. Appendix 1 lists the applicable environmental laws and regulations for each site, Appendix 2 provides occupational exposure information, and Appendix 3 contains the Material Safety Data Sheets (MSDS) for trenbolone acetate and estradiol benzoate.

A. Introduction From Drug Substance Production Sites

(1) Syntex Chemicals, Inc. - Boulder, Colorado

Trenbolone acetate drug substance will be manufactured at this facility.

a. Air Emissions

All emissions of volatile organic compounds generated during the production process are controlled by condensers. In addition, process equipment vents are manifolded and ducted through wet scrubbers employing caustic scrubbing solutions, and finally through activated carbon adsorption units before discharge to the atmosphere. Consequently, the treatment of emissions reduces or eliminates the discharge of these gases into the environment.

b. Wastewater

Aqueous discharges from processing operations are pumped to an on-site aerobic biological wastewater treatment facility to reduce the chemical oxygen demand. Routine monitoring of incoming waste streams consists of the measurement of pH, flammability, and toxic organic compounds. Sludge from the primary clarifier is sent to a permitted hazardous waste landfill. Treated wastewater is then discharged to the City of Boulder's wastewater treatment system. The discharge of treated wastewaters into the City of Boulder's publicly owned treatment works (POTW) system is governed by City of Boulder Ordinance No. 4667.

c. Hazardous Chemical Wastes

Spent solvent streams generated in the trenbolone acetate manufacturing process are recovered and analyzed to determine compliance with appropriate specifications. If they comply, the solvents are reused in the process. Unusable solvents are sent off-site to a permitted hazardous waste treatment facility.

d. Solid Wastes

Diatomaceous earth filter cake also containing silica, carbon, and sodium sulfate will be generated annually from the manufacture of trenbolone acetate. This waste is sent to a permitted hazardous waste landfill.

e. Occupational Exposure

All occupational exposure will be within current regulatory or internal guidelines established for trenbolone acetate and process materials used in the manufacturing process. Occupational exposure information applicable to the manufacture of the drug substance is in Appendix 2.

f. Summary

The pollution control devices in place and the waste disposal methods used by the facility serve to minimize release of environmental emissions resultant from the production of trenbolone acetate. Emissions from this facility are in compliance with the applicable laws and regulations listed for the Boulder site in Appendix 1.

The potential for aquatic toxicity due to the discharge of aqueous waste containing trenbolone acetate was evaluated with a preliminary 48-hour acute *Daphnia magna* toxicity study. Based on immobility and other abnormal effects at 48-hours, the LC_{50} for trenbolone acetate was estimated to be 1,340 ppb (reported as 1.34 mg/L) by the probit estimation method, see Appendix 4. Reduction of aquatic toxicity resulting from manufacturing release would occur by rapid photoreaction, where the half-lives vary from 1.1 to 2.5 hours, see Appendix 5.

(2) Syntex, S.A. de C.V. - Cuernavaca, Mexico

Trenbolone acetate and estradiol benzoate drug substances will be manufactured at this facility.

a. Air Emissions

The Secretaria de Desarrollo Social (SEDESOL) has the regulatory authority for federal implementation of air programs under the Air Pollution and Control Regulations, published in Diario Official de la Federacion on November 25, 1988. SEDESOL issues operational permits to new or existing stationary sources and Syntex, S.A. de C.V. Chemical Division (Syntex Cuernavaca) submitted the application form to SEDESOL to obtain the operational licenses for site activities on May 8, 1992.

Syntex Cuernavaca submitted a general air emissions inventory on September 8, 1989, as well as the results of the air monitoring program on October 8, 1990, which shows the complete compliance with the SO_X (sulfur oxide species), NO_X (nitrogen oxide species), CO and particulate standards applicable to stationary sources. On March 31, 1992, the site presented an emissions control project to SEDESOL which will reduce over 90% of process air emissions.

b. Wastewater

The Mexican Wastewater Regulations, SEDESOL and the Comision Nacional del Agua (CNA) require the control of individual waste discharges including discharges from chemical and pharmaceutical manufacturing operations. In the Cuernavaca Industrial Park (CIVAC), where this Syntex facility is located, the authority for this control has been delegated through the Community Wastewater Treatment Facility (ECCACIV). Quality of wastewater discharged into the ECCACIV collector is controlled under the ECCACIV wastewater treatment service contract. This contract requires all industrial effluents to meet specific standards. The ECCACIV facility has primary and secondary treatment capabilities and operates under SEDESOL surveillance and permit. Every four months Syntex submits a report to ECCACIV on the wastewater quality discharged to the ECCACIV facility.

c. Hazardous Chemical Wastes

Syntex Cuernavaca is a generator of hazardous waste according to Mexican Hazardous Wastes Regulations enacted on November 25, 1988 and disposes of hazardous wastes in accordance with those regulations. Most hazardous waste streams generated by production of the drug substance will be incinerated in an on-site approved hazardous waste incinerator. Some solid wastes are sent to an off-site controlled landfill.

The appropriate federal manifest is issued to ensure each waste shipment arrives at the designated waste facility. All wastes are packed and transported by federally licensed hazardous waste haulers in accordance with hazardous materials transportation regulations.

Biannually a report is submitted to SEDESOL describing the hazardous waste management including handling, storage, treatment and disposal. A general hazardous waste generation inventory was submitted to SEDESOL on July 31, 1989.

d. Solid Wastes

Nonhazardous solid wastes generated by drug substance manufacture are hauled off-site for disposal in an approved landfill.

e. Occupational Exposure

All occupational exposure will be within current regulatory or internal Syntex guidelines established for estradiol benzoate, trenbolone acetate, and process materials used in the manufacturing process. In addition, Material Safety Data Sheets are available for all hazardous chemicals handled at the site. See Appendix 2 for occupational exposure information applicable to the manufacture of the drug substances.

f. Summary

The pollution control devices in place and the waste disposal methods used by the facility serve to minimize release of environmental emissions resultant from the production of trenbolone acetate and estradiol benzoate. Emissions from this facility are in compliance with the applicable laws and regulations listed for the Cuernavaca site in Appendix 1.

B. Introduction From Drug Product Production Sites

(1) Syntex Laboratories, Inc. - Palo Alto, CA

Synovex® Plus (trenbolone acetate and estradiol benzoate) Implant drug product will be manufactured at this facility.

a. Air Emissions

All emissions of particulate matter generated during the production processes are controlled by a dust collection system. Dust particles removed by the baghouse is disposed of as pharmaceutical waste. The baghouse and all the sources abated by it are permitted by the Bay Area Air Quality Management District.

b. Wastewater

Aqueous wastes from production operations are discharged to the Palo Alto Water Quality Control Plant under Industrial Waste Discharge Permit #91135. The wastewater is monitored monthly for total toxic organics and cyanide, and continuously for pH.

c. Hazardous Chemical Wastes

Syntex Palo Alto is a generator of hazardous waste under RCRA and disposes of hazardous waste in accordance with federal and state regulations. All solvent waste streams are recycled or incinerated at approved off-site hazardous waste facilities. Chemical reagents from the laboratories are incinerated at approved off-site hazardous waste facilities. All wastes are packaged and transported by federal and state licensed hazardous waste haulers in accordance with hazardous materials transportation regulations (49 CFR 171-177).

d. Solid Wastes

Nonhazardous solid wastes generated during manufacture, and other facility wastes, are transported by the Palo Alto Sanitation Company to the Palo Alto landfill. All pharmaceutical waste is packaged and shipped for incineration at an approved incineration facility.

e. Occupational Exposure

All occupational exposure will be within current regulatory or internal Syntex guidelines established for estradiol benzoate, trenbolone acetate, process materials, and excipients used in the process. In addition, Material Safety Data Sheets are available for all hazardous chemicals handled at the site. See Appendix 2 for occupational exposure information applicable to the manufacture of the drug product.

f. Summary

The pollution control devices in place and the waste disposal methods used by the facility serve to minimize release of environmental emissions resultant from the production of Synovex® Plus Implant product. Emissions from this facility are in compliance with the applicable laws and regulations listed for the Palo Alto site in Appendix 1.

C. Introduction From Drug Product Use

Estradiol is a naturally occurring hormone which is synthesized in the reproductive glands and/or adrenal glands of all mammals. It has been established by the approvals of NADA 140-897 (Revalor® S - Hoechst-Roussel Agri-Vet) and NADA 118-123 (Compudose® 200 - Elanco Animal Health), that the use of a 24 mg dose of estradiol in feedlot steers is not expected to have a significant impact on the environment. As estradiol benzoate is metabolized to estradiol and estradiol metabolites, the approval of an implant containing 28 mg of estradiol benzoate (equivalent to 20.26 mg of estradiol) is also not expected to have a significant impact on the environment.

Therefore, the discussion below addresses only the potential environmental impact of the use of 200 mg of trenbolone acetate in the Synovex® Plus Implant product in steers fed in confinement for slaughter. The use of 200 mg of trenbolone acetate in heifers fed in confinement for slaughter is already approved and marketed under NADA 138-612.(1)

(1) Metabolism in Steers and Heifers

The potential environmental impact following the use of 200 mg trenbolone acetate in feedlot heifers has been evaluated in an earlier action.(1) The similarity arising from the use of a hormonal implant product containing trenbolone acetate in steers and heifers is demonstrated by comparing tissue residue levels of trenbolone acetate and/or its metabolites in various steer and heifer tissues. A comparison of the trenbolone acetate tissue residues in steers and heifers fifteen and thirty days post-implantation is provided by information in Syntex Document RS-95921 ATv 5940. In this study, equal numbers of steers and heifers were implanted with a nominal dose of 300 mg of ^{14}C-labeled trenbolone acetate per animal (actual dose was 285 mg). Fifteen- and thirty-day post-implantation residue levels in liver, kidney, muscle, fat and bile are presented in Table 1. There are no meaningful differences in the tissue residue levels for steers and heifers at either time point, indicating that steers and heifers metabolize trenbolone acetate and excrete trenbolone acetate residues in essentially the same manner.

The tissue residue levels (steers and heifers) of trenbolone acetate and/or its metabolites arising following the use of 285 mg of trenbolone acetate, as reported in Syntex Document RS-95921 ATv 5940, may be compared to the tissue residue levels reported earlier for implants containing 200 mg trenbolone acetate.(2) Because the latter report does not provide separate tissue residue levels for steers and heifers, the tissue residue levels from Table 1 are combined in Table 2 for more direct comparison. The means and standard deviations in

Table 2 for the combined tissue residue levels were calculated from information reported in Syntex Document RS-95921 ATv 5940.

The data presented in Tables 1 and 2 demonstrate that mean tissue residue levels in liver, kidney, muscle, fat, and bile following the use of 285 mg of trenbolone acetate in steers and heifers are not meaningfully different from the tissue residue levels reported earlier for animals treated with 200 mg trenbolone acetate.[2] Consequently, it is expected that steers will metabolize 200 mg of trenbolone acetate and excrete trenbolone acetate residues in essentially the same manner as heifers metabolize 200 mg of trenbolone acetate and excrete trenbolone acetate residues.

Table 1

Concentrations (Mean) of ^{14}C-Residues in Tissue Collected from Steers and Heifers at 15 and 30 Days after Implantation of ^{14}C-Trenbolone Acetate and Estradiol Benzoate

Syntex Document RS-95921 ATv 5940

	ppb Equivalents of Trenbolone Acetate in Tissue*			
	15 Days		30 Days	
Tissue Sample	Steers	Heifers	Steers	Heifers
Liver	49.3(1)	64.9(1)	85.2(2)	62.0(2)
Kidney	14.2(3)	16.7(3)	21.4(4)	16.1(4)
Muscle	1.12(5)	1.06(5)	19.8(6)	14.5(6)
Fat	1.19(7)	1.67(7)	1.63(8)	1.63(8)
Bile	693(9)	1465(9)	1372(9)	570(9)

(1) Pooled standard deviation (steers and heifers) is 14.0
(2) Pooled standard deviation (steers and heifers) is 34.0
(3) Pooled standard deviation (steers and heifers) is 3.47
(4) Pooled standard deviation (steers and heifers) is 6.77
(5) Pooled standard deviation (steers and heifers) is 0.36
(6) Pooled standard deviation (steers and heifers) is 0.67
(7) Pooled standard deviation (steers and heifers) is 1.06
(8) Pooled standard deviation (steers and heifers) is 1.32
(9) Concentrations expressed as ng equivalents trenbolone acetate per ml (assuming a density of 1.0, ng/mL is equivalent to parts to billion, ppb)
* ppb is equivalent to ng/g as reported

Table 2

Concentrations (Mean and S.D.) of ^{14}C-Residues in Tissue Collected from Cattle at 15 and 30 Days after Implantation of ^{14}C-Trenbolone Acetate and Estradiol Benzoate

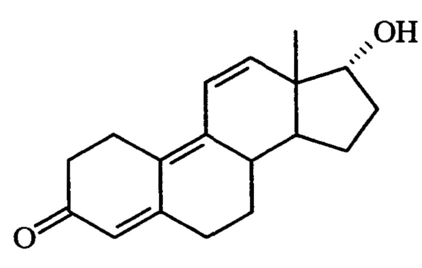
Syntex Document RS-95921 ATv 5940

	ppb Equivalents of Trenbolone Acetate in Tissue*			
	15 Day		30 Day	
Tissure Sample	Mean	S.D.	Mean	S.D.
Liver	58.4	14.0	73.6	34.0
Kidney	15.5	3.47	18.8	6.77
Muscle	1.11	0.36	1.72	0.67
Fat	1.43	1.06	1.63	1.32
Bile (1)	1079	1212	971	1010

(1) Concentrations expressed as ng of trenbolone acetate per ml (assuming a density of 1.0, ng/mL is equivalent to ppb
* ppb is equivalent to ng/g as reported

(2) Estimated Concentration of 17α-Trenbolone in Excreted Cattle Waste

The concentration of 17α-trenbolone, the major metabolite of trenbolone acetate, in the feces of steers may be estimated from the information provided in Syntex Document RS-95921 ATv 5940.(3) 17α-trenbolone is sometimes incorrectly referred to as 17α-hydroxy trenbolone or 17α-OH trenbolone.



Steers implanted with 285 mg of radiolabeled trenbolone acetate produced feces containing an average of 348.5 ppb (reported as ng of trenbolone acetate per gram of feces) as various metabolites.[4] No trenbolone acetate was recovered in the waste.[5] The 17α–trenbolone metabolite accounted for an average of 47.2% of the recovered radioactivity. A 17α–trenbolone glucuronide metabolite accounted for 6.2% of the recovered radioactivity. The combined amount of 17α–trenbolone is 53.4%, expressed as trenbolone acetate, or (53.4%)(270.4/312) = 46.3% expressed as 17α–trenbolone.

The daily urinary excretion of radioactivity (both steers and heifers) averaged the equivalent of 131 ppb, approximately 20% of the amount excreted in feces.[6] However, the percent of 17α–trenbolone in urine was not determined in this study, so the combined amount of 17α–trenbolone in urine and feces cannot be estimated.

For the purpose of this environmental assessment, it is assumed the total amount of trenbolone acetate in the implant is excreted as 17α-trenbolone, even though this is not the case. The combined concentration, (348.5 + 131) or 479.5 ppb, expressed as trenbolone acetate is equivalent to 416 ppb of 17α-trenbolone. Correction for a 200 mg implant brings the estimated level of 17α-trenbolone in cattle waste from animals treated with 200 mg trenbolone acetate implants to (416)(200)/(285) or 292 ppb.

7. ENVIRONMENTAL FATE OF RELEASED SUBSTANCES

As indicated in Section 6, the use of an implant containing 28 mg of estradiol benzoate (equivalent to 20.26 mg of estradiol) in feedlot steers is not expected to have a significant impact on the environment. Metabolism and excretion studies show that trenbolone acetate is rapidly metabolized and has not been recovered in cattle wastes.[5] The major excreted metabolite is 17α-trenbolone, either as free 17α-trenbolone or as its glucuronide conjugate.

Estimation of the environmental distribution and fate of 17α-trenbolone depends upon its chemical and physical properties, including water solubility, vapor pressure, n-octanol/water partition coefficient, and the soil organic carbon/water partition coefficient. The n-octanol/water partition coefficient, K_{OW}, is an expression for the thermodynamic equilibrium constant reflecting the distribution of a chemical between n-octanol and water. K_{OW} has been correlated to hydrophobicity or to the tendency of a chemical to partition out of an aqueous phase into an organic phase. The soil organic carbon/water partition coefficient, K_{OC}, is an expression for the thermodynamic equilibrium constant reflecting the distribution of a chemical between soil organic carbon and water. K_{OC} is thus a measure of the tendency of a chemical to sorb to organic carbon present in soils (or sediments, fecal waste, sludge, etc.) or to partition out of an aqueous phase into soil. If the fraction of organic carbon,

F_{OC}, is known for a soil, an estimate of the equilibrium distribution of a chemical between the soil phase and an aqueous phase may be made as follows:

$$K_{OC} = C_{organic\ carbon} / C_{water} = [(C_{soil} / F_{OC}) / C_{water}]$$

The chemical properties used in subsequent estimations in this Environmental Assessment, see Appendix 6, are presented below.

Water Solubility	= 360 g/m^3 (equivalent to 360,000 ppb)
K_{OW}	= 510
K_{OC}	= 420 (loam soil)
K_{OC}	= 477 (sandy loam soil)
K_{OC}	= 1100 (clay soil)
Vapor pressure	= 4×10^{-10} Torr
	= 5.46×10^{-8} Pa
Melting point	= 92-94.3°C (USP 1a)

The chemical properties given above are derived from the earlier environmental assessment of the use of 200 mg of trenbolone acetate in cattle(1) with two exceptions. The melting point for 17α-trenbolone, 92-94.3°C, was measured by Syntex. The water solubility value reported here for 17α-trenbolone, 360 g/m^3, was measured by Syntex, and is higher than the 40-42 g/m^3 reported earlier.(7) The higher water solubility value reported here is consistent with known relationships between water solubility and octanol/water partition, water solubility and organic carbon/water partition, and the ratios of water solubilities to melting points for the 17α-trenbolone and trenbolone (183-186°C, Merck Index).

A. Estimation of 17α–Trenbolone Levels in Dry/Degraded Waste

Cattle feedlot waste is normally allowed to accumulate on the feedlot, or retained for a period of time prior to disposal. During this time, a large amount of water is lost, as well as some carbon in the form of carbon dioxide produced by microbial activity. The degree of concentration of drug substance over this interval may be estimated from cited values for the average amount of wet feces, 21.8 kg/day, and dry/degraded feces, 3.60 kg/day, produced on average for animals weighing 800 lb.(8) The corresponding concentration of 17α-trenbolone in dry/degraded waste is therefore

$$C_{waste} = (292\ ppb)(21.8 / 3.60)$$

$$= 1{,}768\ ppb$$

This is the estimated concentration in a solid matrix that is approximately 70% organic matter, based on the average ratio of volatile solids to dry solids[9] in manure excreted by feedlot animals.

B. Estimation of 17α–Trenbolone Residue Levels in Feedlot Runoff

The concentration of 17α–trenbolone residues in combined waste from animals treated with 200 mg trenbolone acetate implants has been estimated to be 1,768 ppb. The maximum concentration of 17α–trenbolone that may be reached in water if equilibrium is attained with the accumulated fecal waste may be estimated in two ways.

First, an estimate of a runoff concentration is provided by the known partition between organic carbon and water, K_{OC}. Values of K_{OC} ranging from 420 to 1100 are reported from earlier studies.[10] The decimal fraction organic matter content of dry-degraded waste, 0.70, is equivalent to an organic carbon fraction, F_{OC}, of 0.37.[9] As shown below, the equilibrium concentration that can be reached by partition into water is 11.4 ppb at the minimum value of K_{OC} , 420.

$$K_{OC} = C_{\text{organic carbon}} / C_{\text{water}} = [(C_{\text{waste}} / F_{OC}) / C_{\text{water}}]$$

$$420 = (1{,}768 \text{ ppb})(1.00/0.37) / (C_{\text{water}} \text{ ppb})$$

$$C_{\text{water}} = 11.4 \text{ ppb}$$

The sensitivity of the estimate of runoff concentration to changes in organic carbon content, F_{OC}, and to K_{OC} is shown in the table below. Here, the lower organic carbon content approximates the carbon content of fresh fecal waste.[11]

	$K_{OC} = 420$	$K_{OC} = 1100$
$F_{OC} = 0.20$	21.0 ppb	8.0 ppb
$F_{OC} = 0.37$	11.4 ppb	4.3 ppb

Clearly, the estimated maximum runoff concentration based on simple K_{OC} measurements is less than 21 ppb. These estimates are slightly less than the 37.1 ppb level estimated earlier[12] for runoff from the waste accumulated from animals treated with 200 mg trenbolone acetate.

The second method for estimating runoff levels is a more comprehensive approach, taking into account environmental variables such as amount of rainfall, amount of waste accumulated on the lot surface, amount of accumulated chemical residue(s), and the relative size of air, water and solid waste compartments. The distribution is estimated from known or calculated values of the organic matter content of waste and the partition coefficients between air/water and water/organic carbon (K_{OC}). While one can calculate the equilibrium concentrations of 17α–trenbolone in the environmental compartments by an iterative process, it is much simpler to use the Mackay level 1 computer program.[13] See Appendix 6 for more detailed information on the use of this program.

Runoff concentrations of 17α–trenbolone from one acre of water-saturated feedlot waste four inches in depth with representative organic matter contents are shown in the following table. The 17α–trenbolone loading is based on the complete conversion of the 200 mg trenbolone acetate in each implant to 17α-trenbolone. The table below shows concentrations of 17α–trenbolone when approximately three inches of rain falls on the feedlot, these concentrations are not changed even when the rainfall is increased to six inches (Appendix 6). Clearly, varying the K_{OC} or F_{OC} results in a significant difference in the concentration of 17α-trenbolone in the runoff.

Estimation of 17α-trenbolone in Feedlot Runoff - Mackay Fugacity Model

	Rainfall, 325 $m^3 \approx$ 3"	
	K_{OC} = 420	K_{OC} = 1100
F_{OC} = 0.20	3.3 ppb	1.3 ppb
F_{OC} = 0.37	1.8 ppb	0.7 ppb

The estimates for the 17α-trenbolone concentration in runoff by this environmental model are generally lower than those obtained by the simplistic K_{OC} calcuation. This is in part due to the partition of a given amount of 17α-trenbolone between representative amounts of feedlot waste and rainfall, rather than unit amounts of waste and water. Clearly, most of the excreted 17α-trenbolone is retained with the feedlot waste, with only a small fraction transferred to leachate.

C. Estimation of 17α–Trenbolone Residue Levels in Agricultural Land Resulting from Feedlot Disposal

Feedlot waste is frequently applied to agricultural land as a means of waste disposal. Rates of application are limited by the high salt content of waste, but are generally not in excess of the equivalent of 10 tons (20,000 lbs) dry/degraded waste per acre (the waste may be applied in different forms including a wet slurry). After application, the waste is normally tilled into the top four to six inches of soil. Using an average soil density of 110 lbs/ft^3 (range 100 to 118 lbs/ft^3), the weight of soil in the top four inches of one acre is

$$W_{soil} = (43560\ ft^2/acre)(0.33\ ft)(110\ lbs/ft^3)$$

$$W_{soil} = 1{,}581{,}000\ lbs$$

The corresponding concentration of 17α–trenbolone in soil is then

$$C_{soil} = (1{,}768\ ppb)(20{,}000\ lbs)\ /\ (1{,}581{,}000\ lbs)$$

$$= 22.4\ ppb$$

This level is considerably higher than the earlier estimate, 1.7 ppb, for the application of manure from animals implanted with 200 mg trenbolone acetate.[14] The difference is largely due to the higher concentration of 17α-trenbolone in dry/degraded feedlot waste compared to fresh excreta.

D. Environmental Fate of 17α-Trenbolone Residues

Two expert groups were commissioned to evaluate the potential for transformation of 17α-trenbolone in the environment. The first evaluation was performed at the Environmental Sciences Center at Syracuse Research Corporation. Two QSAR models that divide chemicals into "fast" and "slow" biodegraders gave equivocal results, due to a lack of data for compounds having steroidal ring structures.

The second evaluation, performed at the Center for Biocatalysis and Bioprocessing, University of Iowa, predicted the potential route(s) for environmental biotransformation of 17α-trenbolone. Potential microbial transformations are drawn from known microbial transformations of structurally related steroids. Several pathways appear to be available to generate a lactone intermediate and oxidation products thereof. Many of the proposed biotransformation products should be observable through HPLC analysis. See Appendix 7 for more detailed information.

A study was then performed by Syntex Research to evaluate the potential for aquatic biotransformation of 17α-trenbolone by organisms extracted from field soil. A soil sample was mixed with an equal portion of water to suspend indigenous organisms and the mixture was allowed to settle to remove coarse material. After filtration to remove debris, the suspension was allowed to settle overnight. A portion of the supernatant was withdrawn and transferred to an Erlenmeyer flask containing a known amount of 17α-trenbolone. A positive control flask was prepared using water instead of supernatant from the soil suspension. An additional portion of soil suspension was reserved as a negative control to show the absence of chemicals that interfere in the subsequent analysis of the parent, 17α-trenbolone, and its biotransformation products.

The extent of sorption of 17α-trenbolone to residual suspended solids (clay) was evaluated by withdrawing a sample for analysis at one hour after the start of equilibration. The residual clay in the sample was removed by filtration prior to HPLC analysis. More than 90% of the test compound initially added remained in solution. Samples were then analyzed over a three week period, during which time the 17α-trenbolone disappeared completely. The major biotransformation product was tentatively identified as estra-4,9,11-trien-3,17-dione. This oxidation product corresponds to the proposed biotransformation product #2, Appendix 6. Two other proposed products, 17α-hydroxyestra-1,4,9,11-tetraen-3-one (#10) and 17α-hydroxyestra-4,9-dien-3-one (#6) were not formed in significant amounts, based on UV absorption spectra of the peaks eluting from the HPLC. A summary of this study is presented in Appendix 8. The buildup and partial decay of the estra-4,9,11-trien-3,17-dione with time is illustrated in the following table.

Area Percentage of Parent for Major Biotransformation Products of 17α-trenbolone in a Suspension of Field Soil Organisms

Day	Hours	%Parent (R.T. 4.8 min)	%Product 1 (R.T. 6.2 min)	% Product(s) (R.T. 3.2 min)
0	1	91.8	0	0
1	23	81.2	3.1	0
1.5	28	81.5	4.0	0
2	47	77.3	7.9	0
3	71	73.3	14.1	1.9
6	143	45.9	46.8	1.8
8	171	6.6	86.8	1.8
11	267	0	85.3	1.9
14	335	0	81.1	2.3
20	479	0	66.5	2.5

The experimental measurement of the rate of mineralization of 17α-trenbolone reported earlier(15) is consistent with the proposed route of biodegradation, and the ready conversion to a "17 keto-trienic" compound within a period of 56 days can be accounted for by the formation of the estra-4,9,11-trien-3,17-dione. Even though yearly agricultural applications of waste may occur as a means of waste disposal, the rates of biotransformation of 17α-trenbolone and Product 1 are expected to prevent their accumulation in soil.

The potential for photochemical transformation of 17α-trenbolone in the aquatic environment was first evaluated by Syntex Research by estimating a photolysis half-life from the corresponding ultraviolet absorption spectrum. Given the lack of dissociable functional groups, half-lives are expected to be independent of pH between pH 5 and 9. The estimated minimum Winter half-life at pH 7 is 15.5 seconds for 17α-trenbolone. A summary of this evaluation is presented in Appendix 9.

An additional study was performed by Syntex Research to measure the rate of photolysis upon exposure of 17α-trenbolone to sunlight. The conditions employed in the experiments were based on recommendations in the Technical Assistance Document Section 3.10. However, a reference actinometer was used only for measuring the environmental half-life of 17α-trenbolone in the study at pH 7. Solutions of 17α-trenbolone at pH 5.0, 7.0 and 9.0 were exposed to sunlight for periods between 2 hours before and 3.5 hours after solar noon.

For 17α–trenbolone at pH 5 and 9, the respective half-lives were estimated by the regression of $\ln(C_0/C_t)$ with time. Cloudy, foggy weather conditions during exposure at pH 5 resulted in an apparent half-life of 12.6 hours. Improved weather for exposure at pH 9 resulted in a half-life of 3.3 hours. There should be no difference in photolytic half-life with pH, so the observed difference is weather related. Weather conditions and variations in solar irradiance were compensated for at pH 7.0 by the use of a ρ-nitroanisole/pyridine actinometer. The corrected environmental Winter half-life for 17α-trenbolone at pH 7.0 is 0.61 days. In all cases, the apparent half-lives were less than one day, regardless of the conditions during exposure. Accordingly, these preliminary experiments demonstrate that 17α-trenbolone will degrade rapidly if released to the surface aquatic environment. A summary of this study is presented in Appendix 10.

A supplemental exposure of 17α-trenbolone in sodium bicarbonate buffer solutions (about pH 8) was made to evaluate the relative toxicity of the photoproducts generated on exposure to light. The sodium bicarbonate buffer system is one of the few that do not interfere in the toxicity measurement by the Microtox™ test procedure. The toxicity evaluation was made with *Photobacterium phosphoreum*, the organism supplied for this test. The results of the study, summarized in Appendix 11, demonstrate that the 17α-trenbolone photoproducts are not as toxic as the parent, since the decrease in the toxicity of the photolyzed solutions parallels the decrease of 17α-trenbolone.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

A. Mammalian Toxicity Studies

The human food safety and target species safety of trenbolone acetate have been considered inder the requirements of the Federal Food, Drug, and Cosmetic Act and are not reconsidered here under the National Environmental Policy Act. Information on human food safety and target species safety may be found in the Freedom of Information Summary for NADA 141-03, and in Sections 7 and 8 of NADA 141-043.

B. Potential Toxicity Effects to Non-target Organisms

(1) Aquatic Ecosystems

A preliminary 48-hour acute *Daphnia magna* toxicity study was conducted using 17α-trenbolone to assess its potential for aquatic toxicity. Based on immobility and other abnormal effects after 48-hours, the LC_{50} was estimated to be 340 ppb (reported as 0.34 mg/L) with a 95% confidence interval of 160 to 700 ppb using the probit estimation method. However, the dose-response curve was atypical, as a result of mortalities being observed over a large concentration range (18.1 to 17900 ppb). This made the results difficult to interpret. Given the flat dose-response for the preliminary study, a definitive study was not conducted. A summary of this preliminary study is given in Appendix 12.

(2) Terrestrial Ecosystems

Both sandy loam and clay loam soils were used in the evaluation of the effect of 17α-trenbolone on carbon cycle and nitrogen cycle organisms.(16) It was concluded that "... 17α-trenbolone and 17β-trenbolone [trenbolone] are not expected to have any biologically significant environmental effects on soil carbon and nitrogen cycling microorganisms ..." when present in soils at levels of 15 or 150 ppb.

C. Potential Effects of Proposed Action on Non-target Organisms

(1) Aquatic ecosystems

The introduction of trenbolone acetate into wastewater as a result of drug substance manufacturing operations is not expected to be significant, and is controlled as outlined in Section 6. Metabolism studies show that cattle implanted with 285 mg of trenbolone acetate do not excrete trenbolone acetate in their waste.(5) Trenbolone acetate is not expected to cause a direct environmental effect because it is not excreted by treated animals. Instead a bioactive metabolite, 17α-trenbolone, is excreted and is the focus of the environmental effects characterization.

The concentrations of 17α-trenbolone, the major metabolite of trenbolone acetate, in the aquatic compartment resulting from product use may be significant, as outlined in Section 7 (B). No matter what method is used here, the estimated 17α-trenbolone concentrations in runoff range from 3 ppb to 21 ppb (for organic carbon fraction, F_{OC} , = 0.2 and organic carbon/water partition coefficient, K_{OC} , = 420). This concentration range is expected to be essentially independent of whether steers or heifers are treated with 200 mg trenbolone acetate implants, as the metabolic routes in these animals are very similar (See Section 6).

A preliminary acute *Daphnia magna* toxicity study was performed to evaluate the potential for aquatic toxicity from the introduction of 17α-trenbolone into the environment. This preliminary study showed that 17α-trenbolone was toxic to *Daphnia magna*, and the mortality increased slowly over a large concentration range (18 to 17900 ppb). There is a potential for 17α-trenbolone to induce acute and possibly chronic aquatic effects.

Previously, one would expect that feedlots containing a high proportion of heifers treated with 200 mg trenbolone acetate would have the greatest potential for producing adverse aquatic effects. Apparently, no incidents of adverse effects are on record in the general literature.(17) Effects that reduce the 17α-trenbolone runoff levels, and hence potential toxicity in the aquatic environment, include 1) dilution, arising from slow discharge of runoff into a receiving water system; 2) sorption, arising from contact with soil or sediment solids; 3) metabolism, arising from contact with soil-borne microorganisms; and 4) degradation, resulting from photoreaction with sunlight. In addition, the Microtox™ data, Appendix 11, indicate that the photodegradation products are expected to be significantly less toxic than 17α-trenbolone. In view of these removal mechanisms, the introduction of 17α-trenbolone at concentrations ranging from 3 to 21 ppb is not expected to have a significant adverse impact on the aquatic environment.

(2) Terrestrial ecosystems

The introduction of 17α-trenbolone into the terrestrial environment results from the common practice of applying treated animal wastes onto agricultural land as a means of waste disposal. The estimated concentration in soil is 22.4 ppb for the disposal at a rate equivalent to 10 tons dry/degraded waste per acre.

The potential terrestrial toxicity is addressed in a study of the effect of 15 and 150 ppb levels of 17α-trenbolone on carbon and nitrogen cycle organisms.(18) Other than possible transient stimulatory effects, 17α-trenbolone is not expected to have any biologically significant environmental effects on soil carbon and nitrogen cycling microorganisms at the expected levels in soil.

The primary change resulting from the use of 200 mg trenbolone acetate implants in steers arises from the increased bioburden from the 17α-trenbolone metabolite. The extent of this increase may be estimated from the projected market acceptance of Synovex® Plus Implants. Market research indicates that approximately 13 million steers per year are treated with implants containing trenbolone acetate (140 mg of trenbolone acetate alone or 120 mg trenbolone acetate in combination with 24 mg estradiol). It is estimated that approval of Synovex Plus® Implants could increase the total number of treated steers to approximately 15 million, of which about 50% could be treated with Synovex Plus® Implants. The potential increase in the total amount of 17α-trenbolone metabolites in the environment as a result of the approval of Synovex Plus® Implants could therefore be

100%(15 million)(120 mg) ÷ [(7.5 million)(120 mg) + (7.5 million)(200 mg)]
or 100%(2,400 x10^6 mg) ÷ (1,560 x 10^6 mg), or 54%.

In spite of the projected increase in the total amount of 17α-trenbolone introduced into the terrestrial environment, the estimated concentration of 17α-trenbolone in soil is not expected to increase above levels which result from the currently approved and marketed use in feedlot heifers. This is due to a relatively constant rate of application of feedlot waste to agricultural land, as well as a limited frequency of application. Moreover, preliminary information from soil biodegradation studies(16), and from soil/water biotransformation studies (Appendix 8), indicate 17α-trenbolone will biotransform in agricultural soils sufficiently fast to prevent accumulation upon repeated application of feedlot waste.

9. USE OF RESOURCES AND ENERGY

The raw materials used in the manufacture of trenbolone acetate, estradiol benzoate, and the Synovex® Plus Implant product are readily available. Production of the drug substance and drug product and the energy use involved therein will not cause a depletion of any natural resources that are in critically short supply. The land used for the production of trenbolone acetate, estradiol benzoate, and the Synovex® Plus Implant product is already committed to the production of these and other pharmaceutical products. We know of no effect that the approval of this NADA (Synovex® Plus Implants) may have upon an endangered or threatened species or upon property listed or eligible to be listed in the National Registry of Historic Places.

10. MITIGATION MEASURES

Syntex Chemicals, Inc., Syntex S.A. de C.V. (Cuernavaca), and Syntex Laboratories, Inc. take all necessary measures (described in Section 6) to achieve compliance with the regulations governing the proposed manufacture of these pharmaceutical products. In light of the information presented, no additional mitigation measures are necessary.

No other potential adverse environmental impact is associated with the manufacture of trenbolone acetate, estradiol benzoate, and Synovex® Plus Implants.

11. ALTERNATIVES TO THE PROPOSED ACTION

The proposed action would not be expected to have any adverse effects on human health or on the environment. Therefore, alternatives to the proposed action do not need to be considered.

12. PREPARERS

The following Syntex personnel are responsible for the preparation of this Environmental Assessment:

Lori Bocchino
Environmental Engineering Administrator
Syntex Environmental Health and Safety
Syntex USA Inc.

Marjorie Samples
Staff Researcher I
Syntex Environmental Health and Safety
Syntex USA Inc.

Paul F. Kopeck
Acting Director
Syntex Agribusiness Regulatory Affairs
Regulatory Agent for Syntex Animal Health
Division of Syntex Agribusiness, Inc.

13. CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate, and complete to the best of his knowledge.

Paul F. Kopeck
Acting Director
Syntex Agribusiness Regulatory Affairs
Regulatory Agent for Syntex Animal Health
Division of Syntex Agribusiness, Inc.

14. REFERENCES

1. Hoechst-Roussel Agri-Vet Co., Finaplix® Environmental Assessment Report, NADA 138-612

2. Ibid., p. 8

3. Syntex Doc # RS-95921 ATv 5940

4. Ibid., p. 68-75 (Table 16)

5. Syntex Doc. # RS-95921 ATv 6724, p. 13

6. Syntex Doc # RS-95921 ATv 5940, p. 60-67 (Table 15)

7. NADA 138-612, Loc cit., p. 3

8. "Development Document for Effluent Limitations Guidelines and New Source Performance Standards - Feedlots Point Source Category," p 58-60, Environmental Protection Agency PB-238 651 (1974)

9. Ibid., p. 60

10. NADA 138-612, Loc cit. p. 13

11. Development Document for Effluent Limitations, Loc cit. p. 58

12. NADA 138-612, Loc cit. p. 14-15

13. Mackay, D., "Multimedia Environmental Models: The Fugacity Approach", Lewis Publishers, Chelsea, Michigan (1992).

14. NADA 138-612, Loc cit., p. 15

15. NADA 138-612, Loc cit., p. 12

16. NADA 138-612, Loc cit., p. 18

17. A literature search was conducted for the environmental toxicity (including aquatic toxicity) of 17α-trenbolone, trenbolone, trenbolone acetate, and other anabolic steroids. No information was available on the environmental toxicology of 17α-trenbolone.

18. NADA 138-612, Loc cit. p. 87

15. APPENDICES

Appendix 1	Applicable Laws and Regulations / Compliance Statements
Appendix 2	Occupational Exposure Data
Appendix 3	Material Safety Data Sheets
Appendix 4	Expanded Summary: Acute Toxicity of Trenbolone Acetate to *Daphnia magna*
Appendix 5	Expanded Summary: Preliminary Aqueous Photolysis of trenbolone acetate
Appendix 6	Use of the Mackay Level 1 Fugacity Program for Estimating the Migration of 17α-Trenbolone into Environmental Compartments
Appendix 7	Expanded Summary: Possible Microbial Transformations of 17α-Trenbolone (17α-hydroxyestra-4,9,11-trien-3-one)
Appendix 8	Expanded Summary: Biodegradation of 17α-Trenbolone (17α-hydroxyestra-4,9,11-trien-3-one) by Organisms Extracted From Soil
Appendix 9	Expanded Summary: Estimation of the Maximum Rate of Photolysis of Trenbolone Acetate and 17α-Hydroxy Trenbolone [17α-Trenbolone] in Aqueous Buffer
Appendix 10	Expanded Summary: Preliminary Aqueous Photolysis of 17α-Trenbolone
Appendix 11	Expanded Summary: Microtox Toxicity Evaluation of Photolyzed Solutions of 17α-Trenbolone
Appendix 12	Expanded Summary: Acute Toxicity of 17α-Hydroxy Trenbolone [17α-Trenbolone] to *Daphnia magna*

APPENDIX 1

Applicable Laws and Regulations/Compliance Statements

BOULDER, COLORADO

FEDERAL LAWS	STATUTE	REGULATION
1. Clean Air Act, as amended.	42 U.S.C. § 7401 et seq.	40 CFR 52
2. Resource Conservation and Recovery Act, as amended.	42 U.S.C § 6901 et seq.	40 CFR 260
3. Water Pollution Control Act, as amended (Clean Water Act).	33 U.S.C. § 1251 et seq.	40 CFR 403,439
4. Occupational Safety and Health Act, as amended.	29 U.S.C. § 651 et seq.	29 CFR 1900-1910
5. Emergency Planning and Community Right-to-Know Act.	42 U.S.C. § 11001 et seq.	40 CFR 372
6. Toxic Substance Control Act.	15 U.S.C. § 2601 et seq.	40 CFR 720
7. Hazardous Materials Transportation Act.	49 U.S.C. § 1801 et seq.	49 CFR 171-177
8. Pollution Prevention Act of 1990	42 U.S.C. § 13101 et seq.	

COLORADO LAWS	STATUTE	REGULATION
1. Colorado Air Quality Control Act	Colo. Rev. Stat. § 25-7-101 et seq.	5 CCR § 1001-2 et seq. 5 CCR § 1001-3 et seq. 5 CCR § 1001-5 et seq. 5 CCR § 1001-8 et seq. 5 CCR § 1001-9 et seq. 5 CCR § 1001-10 et seq.

BOULDER, COLORADO (continued)

COLORADO LAWS	STATUTE	REGULATION
2. Colorado Water Quality Control Act	Colo. Rev. Stat. § 25-8-101 et seq.	5 CCR § 1002-2 et seq. 5 CCR § 1002-3 et seq. 5 CCR § 1002-7 et seq. 5 CCR § 1002-8 et seq. 5 CCR § 1002-18 et seq. 5 CCR § 1002-20 et seq.
3. Colorado Hazardous Waste Act	Colo. Rev. Stat. § 25-15-101 et seq.	6 CCR § 1007-3 et seq.
4. Colorado Hazardous Waste Clean Up Act	Colo. Rev. Stat. § 25-16-101 et seq.	6 CCR § 1007-3 et seq.
5. Colorado Hazardous Waste Substance Incidents Law	Colo. Rev. Stat. § 29-22-101 et seq.	
6. Colorado Solid Waste Disposal Sites and Facilities Law	Colo. Rev. Stat. § 30-20-101 et seq.	6 CCR § 1007-2 et seq.

LOCAL RULES	STATUTE	REGULATION
1. Hazardous Material Transportation	Boulder, CO, Code § 6-7-1 et seq.	City of Boulder Ordinance No. 4967
2. Industrial and Prohibited Wastewater Discharges	Boulder, CO, Code § 11-3-1 et seq.	City of Boulder Ordinance No. 4667
3. Storm Water and Flood Management Utility	Boulder, CO, Code § 11-5-1 et seq.	City of Boulder Ordinance No. 4749
4. Land Use Regulation - Special Review Requirement	Boulder, CO Code § 9-3-1 et seq.	City of Boulder Ordinance No. 4803
5. Ozone Depleting Chemical Regulation	Boulder, CO Code § 6-11-1 et seq.	City of Boulder Ordinance No. 5361

MEXICO

LAWS	REGULATIONS	TECHNICAL STANDARDS
Federal Labor Law (1970) (STPS)	Vapor Generators and Pressure Receptacle Inspection Regulations (1936)	
	General Labor Safety and Hygiene Regulations (1978)	
National Program for Protection of the Environment 1990-1994		
General Health Law (1984)	Sanitary Control Regulations for Business Activities, Products, and Services (1988)	Technical Health (SS) Standards (1988-89) Official Mexican Standards
Ecological Equilibrium and Environmental Protection Law (1988) (SEDUE)	Environmental Impact Regulations (1988)	Ecological Standards CE-OESE Specific Observance in Ecological Systems Ecological Standards Waters Quality Control CE-CCA-001/89 NTE-CCA-030/91 NTE-CCA-031/91
	Air Pollution Control Regulations (1988)	Ecological Technical Standard CCAT Air Pollution Control NTE-CCAT-005/88 NTE-CCAT-007/88 NTE-CCAT-008/88 NTE-CCAT-009/88 CCAM Air Pollution Control Monitoring NTE-CCAM-001/88 NTE-CCAM-002/88 NTE-CCAM-001/91 NTE-CCAM-002/91 NTE-CCAM-003/91 NTE-CCAM-004/91 NTE-CCAM-005/91 Official Mexican Standards "AA"

MEXICO (continued)

LAWS	REGULATIONS	TECHNICAL STANDARDS
	Toxic Waste Regulations (1988)	Ecological Technical Standards CRP Control of Toxic Wastes NTE-CRP-001/88 NTE-CRP-002/88 NTE-CRP-003/88 NTE-CRP-008/88 NTE-CRP-009/88 NTE-CRP-010/88 NTE-CRP-011/89
		Official Mexican Standards "AA"
	Regulations for the Prevention and Control of Water Pollution (1973)	Ecological Technical Standards CCA Water Pollution Control Official Mexican Standards "AA"

PALO ALTO

FEDERAL LAWS	STATUTE	REGULATION
1. Clean Air Act, as amended.	42 U.S.C. § 7401 et seq.	40 CFR 52
2. Resource Conservation and Recovery Act, as amended.	42 U.S.C § 6901 et seq.	40 CFR 260
3. Water Pollution Control Act, as amended (Clean Water Act).	33 U.S.C. § 1251 et seq.	40 CFR 403,439
4. Occupational Safety and Health Act, as amended.	29 U.S.C. § 651 et seq.	29 CFR 1900-1910
5. Emergency Planning and Community Right-to-Know Act.	42 U.S.C. § 11001 et seq.	40 CFR 372
6. Toxic Substance Control Act.	15 U.S.C. § 2601 et seq.	40 CFR 720
7. Hazardous Materials Transportation Act.	49 U.S.C. § 1801 et seq.	49 CFR 171-177
8. Pollution Prevention Act of 1990	42 U.S.C. § 13101 et seq.	

PALO ALTO (continued)

	<u>CALIFORNIA LAWS</u>		
1.	California Air Pollution Control Laws and Regulations.	Health and Safety Code 39000 et seq.	CCR Title 17
2.	Air Toxics "Hot Spots" Information Assessment Act.	Health & Safety Code 44300 et seq.	N/A
3.	California Water Quality Laws and Porter-Cologne Act.	Water Code 13020 et seq.	CCR Title 23
4.	Safe Drinking Water and Toxic Enforcement Act.	Health & Safety Code 25249 et seq.	CCR Title 22
5.	Occupational Safety and Health Act	Labor Code 6300 et seq.	CCR Title 8
6.	Hazardous Waste Control Law, as amended.	Health & Safety Code 25100 et seq.	CCR Title 22
7.	Carpenter-Presley-Tanner Hazardous Substance Account Act.	Health & Safety Code 25300 et seq.	N/A
8.	California Solid Waste Management and Resource Recovery Act.	Pub. Res. Code 40000 et seq.	CCR Title 14
9.	Injury and Illness Prevention Program	Labor Code 6401.7	CCR Title 8 Section 3203
10.	Hazardous Waste Reduction, Recycling, and Treatment	Health & Safety Code 25244 et seq.	N/A
11.	Hazardous Materials Release Response Plans and Inventory	Health & Safety Code 25500 et seq.	N/A
	<u>LOCAL LAWS</u>	STATUTE	REGULATION
1.	Bay Area Air Quality Management District Rules and Regulations.	N/A	Bay Area Air Quality Management District Rules and Regulations
2.	City of Palo Alto Hazardous Material Storage Ordinance.	PA Municipal Code Title 17	N/A
3.	City of Palo Alto Municipal Code, Industrial Wastes.	PA Municipal Code Chapter 16	N/A

Compliance Statements

The compliance statements for the manufacturing sites of trenbolone acetate, estradiol benzoate, and the drug product follow.

SYNTEX CHEMICALS, INC.
2075 NORTH 55TH STREET
BOULDER, COLORADO 80301-2880

(303) 442-1926
TELEX 4-5794
FAX: (303) 938-6413

SYNTEX

STATEMENT OF COMPLIANCE

Syntex Chemicals, Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Trenbolone Acetate drug substance at its facilities in Boulder, Colorado as well as emission requirements set forth in applicable federal, state and local statutes and regulations, applicable to the production of Trenbolone Acetate drug substance at its facilities in Boulder, Colorado.

Gerald L. Hoerig
President
Date: 26 JAN 95

SYNTEX, S.A. DE C.V., DIVISION QUIMICA
APARTADO POSTAL 517
62000 CUERNAVACA, MORELOS, MEXICO

TEL. (73) 20 - 24 - 60
FAX (73) 20 - 27 - 54

MARIO LAISECA V., GERENTE GENERAL

Syntex, S.A. de C.V., Division Quimica states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Estradiol Benzoate drug substance and Trenbolone Acetate drug substance at its facilities in Cuernavaca, Morelos, Mexico, as well as emission requirements set forth in applicable federal, state and local statutes and regulations, applicable to the production of Estradiol Benzoate drug substance and Trenbolone Acetate drug substance at its facilities in Cuernavaca, Morelos, Mexico.

Mario Laiseca
General Manager

January 23, 1995

MEMORANDUM

January 24, 1995

TO: Whom it May Concern

FROM: Allen Herd

SUBJECT: **STATEMENT OF COMPLIANCE FOR EB/TBA**

Syntex Laboratories, Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees, and administrative orders applicable to the production of Synovex® Plus (Trenbolone Acetate/Estradiol Benzoate) at its facilities in Palo Alto, California as well as emission requirements set forth in applicable federal, state, and local statutes and regulations, applicable to the production of Synovex® Plus (Trenbolone Acetate/Estradiol Benzoate) at its facilities in Palo Alto, California.

Allen K. Herd

Dr. Allen K. Herd
Vice President, Operations
Syntex Laboratories, Inc.

1/24/95
Date

APPENDIX 2

Occupational Exposure Data

All occupational exposure will be within current regulatory or internal guidelines established for trenbolone acetate, estradiol benzoate, process materials, and excipients used in the manufacturing process. Based on the toxicological profile of the active drug substance, Syntex has established an internal Occupational Exposure Limit (OEL) of:

0.05 mg per cubic meter of air (0.05 mg/m^3) for trenbolone acetate
0.15 mg per cubic meter of air (0.15 mg/m^3) for estradiol benzoate

as an 8-hour time-weighted average. There are no governmental or other regulatory agency exposure levels for estradiol benzoate and trenbolone acetate. The Syntex OEL for these compounds was set to prevent systemic effects.

Appropriate engineering controls will be used to maintain exposures of process materials and excipients below applicable regulatory limits and below the Syntex OEL. If engineering controls are not sufficient, approved respiratory protection will be required which will be in compliance with applicable respiratory protection standards.

Personnel working in the plant are provided with safety helmets, eye protection, uniforms, safety shoes and protective gloves. If conditions warrant, the operators use respiratory protection and additional protective clothing. Directions are written at each appropriate step of the operating procedure advising the operators what safety equipment must be used during that step of the operation. Each operating procedure includes a Safety and Health section advising the operator of potential hazards of all chemicals used in that operation.

APPENDIX 3

MATERIAL SAFETY DATA SHEETS

The material safety data sheets for trenbolone acetate and estradiol benzoate follow.

Material Safety Data Sheet

SYNTEX

Date: July 21, 1994
Supersedes: May 24, 1994
Expires: N/A

SECTION 1. COMPANY AND MATERIAL IDENTIFICATION

Supplier of Data: SYNTEX (U.S.A.) INC.
3401 Hillview Avenue
Palo Alto, CA 94303

In case of an emergency, contact: ENV. HEALTH & SAFETY, (415) 855-5050

MATERIAL IDENTIFICATION

Common Name: **TRENBOLONE ACETATE**

Product Name: **TRENBOLONE ACETATE**

Synonym: **RS-95921-007; TBA; 17 BETA-(ACETOLOXY) ESTRA-4,9,11-TRIENE-3-ONE**

SECTION 2. PRODUCT COMPOSITION

Components:	CAS #:	Formula:	Percentage
TRENBOLONE ACETATE	10161-34-9	C20H24O3	100.00%

SECTION 3. HEALTH HAZARDS

WARNING STATEMENT

WARNING: Contains potent sex steroid hormone. Overexposure may cause reproductive system disorders (including affecting fertility), developmental toxicity (causing birth defects), and has been associated with cancer [related to its effects on endogenous (already occurring naturally in the body) hormones]. Avoid ingestion, inhalation, skin contact, and eye contact. Intended for pharmaceutical manufacturing use only.

ROUTES OF ABSORPTION
Inhalation, skin and eye contact, accidental ingestion.

EYE
Not expected to produce eye irritation. Absorption through the eyes may cause systemic effects.

SECTION 3. HEALTH HAZARDS (CONT'D)

SKIN
Not expected to cause skin irritation. Absorption through the skin may cause systemic effects.

SYSTEMIC, REPRODUCTIVE AND DEVELOPMENTAL TOXICITY
Trenbolone acetate is a synthetic steroid possessing androgenic and anabolic properties. In men, overexposure to trenbolone acetate can produce infertility (inhibition of testicular function, decreased sperm count, decreased seminal volume), changes in libido (sex drive) and impotence, and may produce acne and gynecomastia (breast enlargement). In women, overexposure to trenbolone acetate can lead to menstrual irregularities (changes in menstrual flow, spotting and amenorrhea), masculinization (changes in hair distribution, hoarseness or deepening of the voice, male-pattern baldness, acne) and alteration of libido. Although trenbolone acetate is not listed specifically by the State of California under Proposition 65 as a developmental toxicant (causing birth defects), anabolic steroids are listed as developmental toxicants; trenbolone acetate has anabolic steroid properties.

CARCINOGENICITY AND MUTAGENICITY
Trenbolone acetate caused tumors in laboratory animals at sites in the body related to its hormonal effects or metabolism (stimulation of various target tissues). Data on a battery of mutagenicity tests indicate it is not mutagenic (does not affect genetic material). While trenbolone acetate is not specifically listed by NTP, IARC, or OSHA as a carcinogen, androgenic and anabolic steroids, such as trenbolone acetate, are listed by IARC and the State of California (under Proposition 65) as carcinogens.

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE
None known or reported.

PERMISSABLE EXPOSURE LIMIT
None currently established by AGGIH or OSHA. Syntex has developed an Occupational Exposure Limit (OEL) based on the potency of trenbolone acetate compared to testosterone, a naturally occurring androgen. The OEL for trenbolone acetate is 0.05 micrograms/m3 of air as an 8 hour/day time weighted average for a 40 hour work week. The OEL has been developed, with suitable safety factors to reduce the potential for effects on endogenous hormonal levels.

SECTION 4. FIRST AID MEASURES

EYE CONTACT
Immediately flush eyes thoroughly with water. Contact supervisor and medical personnel.

SKIN CONTACT
Immediately wash thoroughly with soap and water. Contact supervisor and medical personnel.

SECTION 4. FIRST AID MEASURES (CONT'D)

INHALATION
Immediately move to fresh air. Contact supervisor and medical personnel.

INGESTION
Give moderate amount of water. Immediately contact supervisor and medical personnel.

SECTION 5. FIRE PROTECTION

FLASH POINT
Not applicable (Solid).

MINIMUM EXPLOSIVE CONCENTRATION
Not determined. As a finely divided solid, should be handled as a combustible powder.

MINIMUM IGNITION ENERGY
Not determined.

EXTINGUISHING MEDIA
Water, multipurpose dry chemical or halon fire extinguisher.

SPECIAL FIRE FIGHTING PROCEDURES
Wear full protective clothing and NIOSH/MSHA approved positive pressure self-contained breathing apparatus. Thoroughly wash all equipment after use.

SECTION 6. SPILL AND RELEASE MEASURES

If material is released or spilled cordon off spill area. For small spills of solids, do not attempt to sweep up dry materials; use water or an appropriate solvent to dilute and clean up. Soak up material with paper towels and wash spill area thoroughly with soap and water. For larger spills, wet down spilled material with water or an appropriate solvent to minimize dust generation. Scoop into suitable containers appropriate for either recovery or disposal to avoid exposure and to comply with applicable waste disposal regulations. Alternatively, use an industrial vacuum cleaner equipped with a high efficiency particulate filter. Remove filter and dispose of it in a manner to avoid exposure and to comply with applicable waste disposal regulations. Take proper precautions to minimize exposure by using appropriate personal protective equipment.

SECTION 7. HANDLING AND STORAGE

Avoid contact with skin, eyes, or clothing. Use adequate ventilation to minimize dust generation. Wash thoroughly after handling. Store in a cool, well-ventilated area.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION

EYE PROTECTION
Wear safety glasses with side shields, chemical splash goggles, or full face shield. The choice of eye protection should be appropriate for the job activity.

RESPIRATORY PROTECTION
Where applicable, this material should be handled in closed processes or containers. If it is properly handled in a glove box, laboratory hood, or with effective local exhaust ventilation or in a closed system, respiratory protection may not be needed. For handling small (milligram) quantities (such as for QC activities), a properly fitted half-mask air purifying respirator with NIOSH/MSHA approval for dusts and mists is recommended. If operations result in exposures up to 10 times the OEL, a properly fitted half-mask air purifying respirator is the minimum respiratory protection required. For exposures greater than 10 times the OEL but less than 50 times the OEL, wear a full face piece air purifying respirator with NIOSH/MSHA approval for dusts and mists. At concentrations greater than 50 times the OEL and for dusty operations handling bulk quantities, e.g., milling, a "hood or helmet type" powered air-purifying respirator or an air supplied respirator is required.

SKIN PROTECTION
Rubber (latex) or other impermeable gloves (if solvent solutions are used, make sure the glove is impermeable to the particular solvent(s)). Tyvek sleeve covers, aprons, lab coats or other impermeable clothing may also be needed, depending on the job activity.

VENTILATION
Effective local exhaust ventilation, controlled handling techniques and isolation of dust emission sources are recommended for processes where dust routinely might be generated.

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES

BOILING POINT: Not applicable
MELTING POINT: 90-95 degrees C
MOLECULAR WEIGHT: 312
SOLUBILITY: Soluble in methanol
VAPOR PRESSURE: Not determined
SPECIFIC GRAVITY: Not determined
PERCENT VOLATILE: Not applicable
VAPOR DENSITY: Not applicable
EVAPORATION: Not applicable
APPEARANCE, COLOR, ODOR: White crystalline solid

SECTION 10. STABILITY AND REACTIVITY

STABILITY
Stable.

SECTION 10. STABILITY AND REACTIVITY (CONT'D)

INCOMPATIBILITY
Avoid contact with strong oxidizers.

HAZARDOUS DECOMPOSITION PRODUCTS
Not determined.

HAZARDOUS POLYMERIZATION
None.

SECTION 11. TOXICOLOGICAL INFORMATION

Trenbolone acetate has activity as an androgen (male reproductive hormone). In laboratory rodents and non-human primates, the following toxicological effects have been reported: atrophy of testis, prostate and seminal vesicles, decrease in spermatazoa numbers, atrophy of mammary gland, dilation of uterine lumen, and alterations in the menstrual cycles. Because of its androgenic properties, this chemical has the potential for causing acne, growth of facial hair, alterations in menstrual cyclicity, and virilization in women. It is considered a developmental toxicant as it may cause virilization (masculinization) effects on female offspring exposed in utero. In long term animal studies, this chemical has been shown to be carcinogenic [probably related to its effects on endogenous (already naturally occurring in the body) hormones].

SECTION 12. ENVIRONMENTAL INFORMATION

Persistence and Degradability
The octanol/water partition coefficient for trenbolone acetate and its major metabolites, 17-alpha trenbolone and 17-beta trenbolone were 5900, 510 and 1040, respectively, suggesting a low potential for bioaccumulation in organisms in the environment. The adsorption/desorption Koc values for 17-alpha trenbolone and 17-beta trenbolone in three soil types ranges from 500 to 9500, suggesting low to medium leachability. Trenbolone acetate metabolites are significantly degraded in the presence of sunlight and by soil microorganisms.

Aquatic Toxicity
No data.

SECTION 13. WASTE DISPOSAL METHODS

All wastes containing the compound should be specially contained, properly labeled, and stored separately from other facility discharges. Dispose of any waste residues according to prescribed federal, state or local guidelines, e.g., appropriately permitted chemical waste incinerator. Rinsewaters resulting from spill cleanups should be discharged in an environmentally safe manner, e.g., appropriately permitted municipal or on-site wastewater treatment facility.

SECTION 13. WASTE DISPOSAL METHODS (CONT'D)

Disposal methods should be used which in addition to preventing environmental contamination, also prevents human exposure to the waste residues. For example, ensure that there will not be any chance for humans to be exposed to the undiluted/active waste residues.

SECTION 14. TRANSPORTATION INFORMATION

Hazard Class
Not regulated or classified by U.S. DOT or IATA
(Note: Pending U.S. FDA approval for Synovex Plus, materials containing trenbolone acetate are regulated under Schedule III of the Controlled Substances Act, 21 U.S.C. 801, et seq., and should be distributed and/or dispensed in accordance with that act.

UN Number
Not assigned

SECTION 15. LABELING/REGULATORY INFORMATION

Bulk containers of trenbolone acetate should have affixed the following warning label (in addition to the material identity label):

WARNING: Contains potent sex steroid hormone. Overexposure may cause reproductive system disorders (including affecting fertility), developmental toxicity (causing birth defects), and has been associated with cancer [related to its effect on endogenous (already occurring naturally in the body) hormones]. Avoid inhalation, ingestion, skin contact and eye contact. Material intended for pharmaceutical manufacturing use only. Read and understand the Material Safety Data Sheet before working with this product.

PROPOSITION 65 INFORMATION
Anabolic steroids are listed by the State of California as carcinogens and reproductive toxicants; trenbolone acetate possesses anabolic steroid activity.

EUROPEAN UNION (EC) RISK PHRASES
R45 May cause cancer
R60 May impair fertility
R61 May cause harm to unborn child

CANADIAN WHMIS
This MSDS meets and provides all the necessary information required under the Canadian regulation (Workplace Hazardous Materials Information System).

SECTION 16. OTHER INFORMATION

SECTION 16. OTHER INFORMATION (CONT'D)

The above information is based on currently available data and to the best of our knowledge is correct. The information is applicable to the intended uses of, as well at the foreseeable emergencies involving this material, but may not pertain to all potential misuses of the material.

No representation, warranty, or guarantee, express or implied (including a warranty of fitness or merchantability for a particular purpose), is made with respect to the material or the results to be obtained from the use thereof. Caution should be used in the handling and use of this material because it is a biologically active compound.

Material Safety Data Sheet

SYNTEX

Date: July 21, 1994
Supersedes: May 26, 1994
Expires: N/A

SECTION 1. COMPANY AND MATERIAL IDENTIFICATION

Supplier of Data: SYNTEX (U.S.A.) INC.
3401 Hillview Avenue
Palo Alto, CA 94303

In case of an emergency, contact: ENV. HEALTH & SAFETY, (415) 855-5050

MATERIAL IDENTIFICATION

Common Name: **ESTRADIOL BENZOATE**

Product Name: **ESTRADIOL BENZOATE**

Synonym: **RS-304-007; ESTRA-1,3,5(10)-TRIENE-3,17BETA-DIOL, 3-BENZOATE; ESTRADIOL-3-BENZOATE**

SECTION 2. PRODUCT COMPOSITION

Components:	CAS #:	Formula:	Percentage
ESTRADIOL BENZOATE	50-50-0	$C_{25}H_{28}O_3$	100.00%

SECTION 3. HEALTH HAZARDS

WARNING STATEMENT

WARNING: Contains potent sex steroid hormone. Overexposure may cause reproductive system disorders (including affecting fertility), developmental toxicity (causing birth defects) and has been associated with cancer [related to its effect on endogenous (already naturally occurring in the body) hormones]. Avoid ingestion, inhalation, skin contact, and eye contact. Material intended for pharmaceutical manufacturing use only.

ROUTES OF ABSORPTION
Inhalation, Skin and Eye Contact, Accidental Ingestion.

EYE
Not expected to produce eye irritation. Absorption through the eyes may cause systemic effects.

SECTION 3. HEALTH HAZARDS (CONT'D)

SKIN
Not expected to cause skin irritation. Absorption through the skin may cause systemic effects.

SYSTEMIC, REPRODUCTIVE AND DEVELOPMENTAL TOXICITY
In men, overexposure to estradiol benzoate can lead to gynecomastia (breast tenderness, breast nodules or enlarged breasts), galactorrhea (secretion of milk), decreasd libido and damage to testes resulting in atrophy and infertility.

In women, the most common effects from overexposure are menstrual irregularities (breakthrough bleeding, changes in menstrual flow, spotting, and amenorrhea), breast changes (tenderness, enlargement, and secretion), and fluid retention.

Estrogens, such as estradiol benzoate, are developmental toxicants (potentially causing birth defects in the offspring of the exposed pregnant female).

CARCINOGENICITY AND MUTAGENICITY
The IARC has determined that there is sufficient evidence that estrogens, which includes estradiol benzoate, are carcinogenic in experimental animals, but that the data are inadequate to evaluate the carcinogenicity in humans. Estrogens, which includes estradiol benzoate, are listed by the NTP as carcinogenic. The carcinogenic activity of estrogens are considered to be related to their effect on endogenous (already naturally occurring in the body) sex hormones and the stimulation of various target tissues by these hormones. Estradiol benzoate is not considered mutagenic (affect genetic material) based on a battery of mutagenicity studies.

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE
Not known or reported.

OCCUPATIONAL EXPOSURE LIMIT
Syntex has established an occupational exposure limit (OEL) of 0.15 microgram/m3 as an 8-hour time-weighted average over a 40 hour work week. The OEL has been developed, with suitable safety factors, to reduce the potential for effects on endogenous hormone levels.

SECTION 4. FIRST AID MEASURES

EYE CONTACT
Immediately flush eyes thoroughly with water for at least 15 minutes and contact medical personnel and supervisor.

SKIN CONTACT
Immediately wash thoroughly with soap and water for 15 minutes. If an irritation develops, contact medical personnel and supervisor.

SECTION 4. FIRST AID MEASURES (CONT'D)

INHALATION
Immediately move to fresh air and contact medical personnel and supervisor.

INGESTION
Give moderate amount (8-12 oz.) of water and immediately contact medical personnel and supervisor.

SECTION 5. FIRE PROTECTION

FLASH POINT
As a powder, not applicable.

FLAMMABILITY LIMITS
As a finely divided solid, should be handled as a combustible powder.

EXTINGUISHING MEDIA
Water, multipurpose dry chemical or halon-fire extinguisher.

SPECIAL FIRE FIGHTING PROCEDURES
Wear full protective clothing and NIOSH/MSHA-approved, positive pressure, self-contained breathing apparatus. Thoroughly wash all equipment after use.

SECTION 6. SPILL AND RELEASE MEASURES

If material is released or spilled cordon off spill area. For small spills of solids, do not attempt to sweep up dry materials; use water or an appropriate solvent to dilute and clean up. Soak up material with paper towels and wash spill area thoroughly with soap and water. For larger spills, wet down spilled material with water or an appropriate solvent to minimize dust generation. Scoop into suitable containers appropriate for either recovery or disposal to avoid exposure and to comply with applicable waste disposal regulations. Alternatively, use an industrial vacuum cleaner equipped with a high efficiency particulate filter. Remove filter and dispose of it in a manner to avoid exposure and to comply with applicable waste disposal regulations. Take proper precautions to minimize exposure by using appropriate personal protective equipment.

SECTION 7. HANDLING AND STORAGE

Avoid contact with skin, eyes, or clothing. Use adequate ventilation to minimize dust generation. Wash thoroughly after handling. Store in a cool, well-ventilated area.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION

EYE PROTECTION
Wear safety glasses with side shields, chemical splash goggles, and/or full face shield to prevent contact with eyes. The choice of protection should be based on the job activity and potential for exposure to the eyes and face.

RESPIRATORY PROTECTION
Where applicable, this material should be handled in closed processes or containers. If it is properly handled in a glove box, laboratory hood, or with effective local exhaust ventilation or in a closed system, respiratory protection may not be needed. For handling small (milligram) quantities (such as QC activities), a properly fitted half mask respirator with NIOSH/MSHA aspproval for dusts and mists is recommended. If operations result in exposures up to 10 times the OEL, a properly fitted 1/2 mask respirator with NIOSH/MSHA (or equivalent) approval for dusts and mists is the minimum respiratory protection required. For exposures greater than 10 times the OEL but less than 50 times the OEL, wear a full face piece air purifying respirator with NIOSH/MSHA approval for dusts and mists. At concentrations greater than 50 times the OEL and for dusty operations handling bulk quantities, e.g., milling, a "hood or helmet type" powered air purifying respirator or supplied air respirator is required.

SKIN PROTECTION
Rubber (latex) gloves are recommended to minimize potential for skin contact when handling the active in dry form or in aqueous solutions. When the compound is dissolved in organic solvent, wear gloves that provide protection against that solvent. Wear lab coat or other protective overgarment. The choice of skin protection should be based on the job activity and potential for exposure to the skin.

VENTILATION
When practicable, the material should be handled in enclosed processes, a properly operating laboratory hood or with other effective local exhaust ventilation.

OTHER
Wash hands, face and other potentially exposed areas immediately after working with this material (especially before eating, drinking, or smoking). All protective equipment should be thoroughly cleaned as well.

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES

BOILING POINT: Not applicable/Solid
MELTING POINT: 190-196 degrees C
MOLECULAR WEIGHT: 376.4
SOLUBILITY: Insoluble in water, soluble in alcohol, ether, acetone, dioxane, and chloroform.
VAPOR PRESSURE: Nil
SPECIFIC GRAVITY: Unknown
PERCENT VOLATILE: Nil
VAPOR DENSITY: Unknown
EVAPORATION: Nil
APPEARANCE, COLOR, ODOR: White to creamy white crystalline powder, odorless.

SECTION 10. STABILITY AND REACTIVITY

STABILITY
Stable.

INCOMPATIBILITY
Strong oxidizing agents (e.g., peroxides, permanganates, nitric acid, etc.) may produce violent reaction.

HAZARDOUS DECOMPOSITION PRODUCTS
Combustion may produce carbon dioxide and carbon monoxide.

HAZARDOUS POLYMERIZATION
Will not occur.

SECTION 11. TOXICOLOGICAL INFORMATION

Estradiol benzoate is a synthetic estrogen (female sex hormone). Estrogens can alter the cyclical response of the menstrual cycle in women and have caused gynecomastia (breast nodules) and other feminizing effects in occupationally exposed men. Estrogens are developmental toxicants as exposure in utero has been associated with limb malformations, effects on developing reproductive organs and transplancental carcinogenesis. In laboratory animal studies, estrogens have been reported to increase the frequency of cancer of the breast, cervix, vagina, testis, bone and liver [possibly related to its effects on endogenous (already naturally occurring in the body) hormones]. In humans, long term estrogen use has been reported to increase the risk of endometrial carcinoma [also possibly related to its effects on endogenous hormones], thromboembolic and thrombotic vascular disease (blood clotting disorders) and retinal thrombosis (bleeding disorder in the eye. The risk is magnified in women over the age of 35 who are smokers.

See Section 3. Health Hazards.

SECTION 12. ENVIRONMENTAL INFORMATION

Persistence and Degradability
No data.

Aquatic Toxicity
No data.

SECTION 13. WASTE DISPOSAL METHODS

All wastes containing the compound should be specially contained, properly labeled, and stored separately from other facility discharges. Dispose of any waste residues according to prescribed federal, state or local guidelines, e.g., appropriately permitted chemical waste incinerator. Rinsewaters resulting from spill cleanups should be discharged in an environmentally safe manner, e.g., appropriately permitted municipal or on-site wastewater treatment facility.

SECTION 13. WASTE DISPOSAL METHODS (CONT'D)

Disposal methods should be used which in addition to preventing environmental contamination, also prevents human exposure to the waste residues. For example, ensure that there will not be any chance for humans to be exposed to the undiluted/active waste residues.

SECTION 14. TRANSPORTATION INFORMATION

Hazard Class
Not regulated

UN Number
Not assigned

SECTION 15. LABELING/REGULATORY INFORMATION

Bulk containers of estradiol benzoate should have affixed the following warning label (in addition to the product identity label):

WARNING: Contains potent sex steroid hormone. Overexposure may cause reproductive system disorders (including affecting fertility), developmental toxicity (causing birth defects) and has been associated with cancer [related to its effect on endogenous (already naturally occurring in the body) hormones]. Avoid ingestion, inhalation, skin contact, and eye contact. Material intended for pharmaceutical manufacturing use only. Read and understand the Material Safety Data Sheet for additional information before working with this product.

PROPOSITION 65 INFORMATION
Estradiol benzoate is not specifically listed by the State of California as a carcinogen or reproductive toxicant. Other estrogenic compounds, with similar properties are listed as carcinogenic and/or reproductive toxicants.

EUROPEAN UNION (EC) RISK PHRASES
R45 May cause cancer
R60 May impair fertility
R61 May cause harm to unborn child

CANADIAN WHMIS
This MSDS meets and provides all the necessary information required under the Canadian regulation (Workplace Hazardous Materials Information Systems).

SECTION 16. OTHER INFORMATION

SECTION 16. OTHER INFORMATION (CONT'D)

The above information is based on currently available data and to the best of our knowledge is correct. The information is applicable to the intended uses of, as well at the foreseeable emergencies involving this material, but may not pertain to all potential misuses of the material.

No representation, warranty, or guarantee, express or implied (including a warranty of fitness or merchantability for a particular purpose), is made with respect to the material or the results to be obtained from the use thereof. Caution should be used in the handling and use of this material because it is a biologically active compound.

APPENDIX 4

Expanded Summary: Acute Toxicity of Trenbolone Acetate to *Daphnia magna*.

Syntex Document # RS-95921 CH 0282

Location of Study:	Toxikon Environmental Sciences 106 Coastal Way Jupiter, FL 33477
Location of Raw Data: and Final Report	Syntex Discovery Research Palo Alto, CA 94304
Test Species:	Water Flea, (*Daphnia magna*)
Source of Organisms:	Toxikon Environmental Sciences Jupiter, FL 33477
Condition at Start of Study:	Daphnids appeared to be in good physical condition at test initiation
Dilution Water:	Treated Jupiter town water with an initial hardness and alkalinity of 66 and 39 mg/L as $CaCO_3$, respectively; test temperature range of 19.8 to 20.7°C.
Nominal (Measured) Concentrations:	Control (<0.005), 0.64 (0.580), 1.06 (0.986), 1.77 (1.61), 2.96 (2.80), 4.93 (4.96) mg a.i./L
Test Dates:	Start; October 4, 1994 Completion; October 6, 1994
Study Length:	48 Hours
Results:	The 48 hour LC_{50} was 1.34 mg a.i./L, based upon measured concentrations. The 95% confidence interval was from 1.07 mg/L to 1.65 mg/L. The NOEC was <0.580 mg a.i./L, based on the significant mortality observed at this and higher test concentrations (15% mortality was observed at the lowest measured concentration of 0.580 mg a.i./L).

APPENDIX 5

Expanded Summary: Preliminary Aqueous Photolysis of Trenbolone Acetate

Syntex Document # RS-95921 CH 0281

The purpose of this study was to evaluate the extent of photolysis during a one day exposure to sunlight under winter conditions. The work was conducted in three parts on three separate days, with each part corresponding to an exposure at a designated pH of the photolyzed solution. For photolysis at a pH of 5, a 2.63×10^{-5} M trenbolone acetate solution was prepared in a 0.1 M ammonium phosphate buffer. For photolysis at pH 7, a 1.47×10^{-5} M trenbolone acetate solution was prepared in 0.1 M sodium phosphate buffer. For photolysis at pH 9, a 3.46×10^{-5} M solution of trenbolone acetate solution was prepared in 0.1 M sodium borate buffer. In each study, both foil wrapped and unwrapped quartz tubes containing trenbolone acetate solution were exposed to sunlight. At 30 minute intervals, one set of tubes consisting of two exposed and one wrapped tube were sampled for analysis by HPLC. As the control tubes indicated negligible nonphotolytic reactions, the photolytic rate constant was calculated from the best fit of $\ln (C_0/C_t)$ versus exposure time, and the half-life calculated from the rate constant.

Trenbolone was found to undergo extensive photolysis in a one day period, with half-lives at pH 5, 7, and 9 of 1.1, 1.3, and 2.5 hours, respectively. The longer half-life at pH 9 was due to periods of rain in the exposure period.

APPENDIX 6

Use of the Mackay Level 1 Fugacity Program for Estimating the Migration of 17α-trenbolone into Environmental Compartments

The Mackay Level 1 computer program(1) may be used to estimate runoff concentrations of 17α-trenbolone, the major metabolite of trenbolone acetate, from accumulated animal waste in a feedlot. The estimation makes use of typical parameters for the number of treated animals per acre of feedlot, the amount of accumulated waste per animal, the water holding capacity of waste, and the amount of rainfall. The distribution is determined by the chemical properties of 17α-trenbolone, as noted below.

Water Solubility	360 g/m^3
K_{OW}	510
K_{OC}	420 (loam soil)
K_{OC}	1100 (clay soil)
Vapor Pressure	5.46×10^{-8} Pa
Melting Point	92 to 94.3°C

The chemical properties presented above are derived from the earlier environmental assessment of the use of 200 mg of trenbolone acetate in cattle(2) with two exceptions: the melting point for 17α-trenbolone (92-94.3°C, Appendix 10); and the water solubility value reported here for 17α-trenbolone, as measured by Syntex, is higher than the 40-42 g/m^3 reported earlier(3). The higher water solubility value reported here is consistent with known relationships between water solubility and octanol/water partition, water solubility and organic carbon/water partition, and the ratios of water solubilities and melting points for the 17α-trenbolone and trenbolone (183-186°C, Merck Index).

The relative size of the environmental compartments must first be established to start the analysis. A surface area of one acre is used here, although any area unit may be selected. With sufficient rainfall, the dry waste becomes saturated and runoff begins. The composition of the surface solids will then approximate fresh animal feces, where about 80% by volume is water and the remainder is solid matter. Assuming runoff may take place from the top four inches of waste in the feedlot, the volume of water available for runoff is 325 m^3/acre, and the volume of solids is 81.4 m^3/acre. This volume of water corresponds to slightly more than 3" of rainfall (308 m^3). The volume of an air column 1000 feet above an acre of feedlot surface is 1.23×10^6 m^3.

The distribution of 17α-trenbolone between water and soil depends not only on the organic carbon partition coefficient, K_{OC}, but also on the fraction organic carbon (or organic matter) in the solid waste. Based on the average ratio of volatile solids to dry solids (4), solid waste may contain approximately 70% organic matter. This corresponds to an organic carbon decimal fraction, F_{OC}, of (0.70 / 1.9) or 0.368. However, based on the minimum organic content of manure(5), the fraction organic carbon may be as low as 0.200. This range of values for the organic carbon content will be used in subsequent calculations to illustrate the degree to which the estimated runoff concentrations depend upon the organic carbon content of feedlot waste.

The total amount of 17α-trenbolone initially introduced into the compartment is not critical unless it exceeds the capacity of the compartment. For the purpose of runoff estimation, we assume an average animal density of 200 animals/acre, and that they excrete an amount of 17α-trenbolone equivalent to all 200 mg trenbolone acetate in the implants. On a per acre basis, this amount is (200 animals/acre)(200 mg/implant)(270.4 / 312) mg, or 34,555 mg.

The Mackay level 1 distribution may now be estimated, using a range of organic carbon fractions, 0.368 to 0.200, a range of organic carbon partition coefficients, 420 to 1100, and rainfall amounts from 325 to 650 m^3, corresponding to approximately 3" to 6" of rain. The runoff concentrations under these conditions are given in the Tables below.

Estimation of 17α-trenbolone in Feedlot Runoff - Mackay Fugacity Model

Rainfall, 325 $m^3 \approx$ 3"

	$K_{OC} = 420$	$K_{OC} = 1100$
$F_{OC} = 0.20$	3.3 ppb	1.3 ppb
$F_{OC} = 0.37$	1.8 ppb	0.7 ppb

Rainfall, 650 $m^3 \approx$ 6"

	$K_{OC} = 420$	$K_{OC} = 1100$
$F_{OC} = 0.20$	3.2 ppb	1.3 ppb
$F_{OC} = 0.37$	1.8 ppb	0.7 ppb

The computer printouts for the estimations using the Mackay fugacity model follow.

References:

1. Mackay, D., "Multimedia Environmental Models: The Fugacity Approach", Lewis Publishers, Chelsea, Michigan (1992).
2. Hoechst-Roussel Agri-Vet Co., Finaplix® Environmental Assessment Report, NADA 138-612
3. NADA 138-612, Loc cit., p 3
4. "Development Document for Effluent Limitations Guidelines and New Source Performance Standards - Feedlots Point Source Category," p 58- 60, Environmental Protection Agency PB-238 651 (1974), p. 60
5. Development Document for Effluent Limitations, Loc cit. p. 58

1. File 17tre3b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 420; F_{OC} is 0.2; rainfall is 3".

2. File 17tre4b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 1100; F_{OC} is 0.2; rainfall is 3".

3. File 17tree9b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 420; F_{OC} is 0.368; rainfall is 3".

4. File 17tre2b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 1100; F_{OC} is 0.368; rainfall is 3".

5. File 17tre5b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 420; F_{OC} is 0.2; rainfall is 6".

6. File 17tre6b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 1100; F_{OC} is 0.2; rainfall is 6".

7. File 17tre7b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 420; F_{OC} is 0.368; rainfall is 6".

8. File 17tre8b.dat — Phase properties and 17α-trenbolone compositions for the water/feedlot/air compartments. Average waste depth of 4" in the lot. Sixty day accumulation of 17α-trenbolone. K_{OC} is 1100; F_{OC} is 0.368; rainfall is 6".

1. File 17tre3b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for 60 day total excretion
B:\17tre3b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	4.20000000000000E+0002
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	1.26000000000000E+0001
Sediment/water partition coefficient	1.26000000000000E+0002

Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	4.95359781308193E-0013
Total of VZ products:	2.58398046894250E+0011

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	3.2500E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	2.0000E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	3.0769E+0008	3.0769E+0009
VZ mol/Pa	4.9620E+0002	7.9365E+0009	0.0000E+0000	2.5046E+0011
Fugacity Pa	4.9536E-0013	4.9536E-0013	4.9536E-0013	4.9536E-0013
Conc mol/m3	1.9984E-0016	1.2097E-0005	1.5242E-0004	1.5242E-0003
Conc g/m3	5.3956E-0014	3.2661E-0003	4.1153E-0002	4.1153E-0001
Conc ug/g	4.5517E-0011	3.2661E-0003	2.7435E-0002	2.7435E-0001
Amount mol	2.4580E-0010	3.9314E-0003	0.0000E+0000	1.2407E-0001
Amount %	0.000000	3.071427	0.000000	96.928573

2. File 17tre4b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total metabolite excretion in 60 days
B:\17tre4b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	1.10000000000000E+0003
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	3.30000000000000E+0001
Sediment/water partition coefficient	3.30000000000000E+0002
Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	1.92798028325392E-0013
Total of VZ products:	6.63907204403408E+0011

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	3.2500E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	2.0000E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	8.0586E+0008	8.0586E+0009
VZ mol/Pa	4.9620E+0002	7.9365E+0009	0.0000E+0000	6.5597E+0011
Fugacity Pa	1.9280E-0013	1.9280E-0013	1.9280E-0013	1.9280E-0013
Conc mol/m3	7.7778E-0017	4.7081E-0006	1.5537E-0004	1.5537E-0003
Conc g/m3	2.1000E-0014	1.2712E-0003	4.1949E-0002	4.1949E-0001
Conc ug/g	1.7715E-0011	1.2712E-0003	2.7966E-0002	2.7966E-0001
Amount mol	9.5667E-0011	1.5301E-0003	0.0000E+0000	1.2647E-0001
Amount %	0.000000	1.195424	0.000000	98.804576

3. File 17tree9b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total excretion of 17a trenbolone
B:\17tree9b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	4.20000000000000E+0002
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	1.26000000000000E+0001
Sediment/water partition coefficient	2.31840000000000E+0002
Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	2.73045848659787E-0013
Total of VZ products:	4.68785739201943E+0011

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	3.2500E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	3.6800E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	3.0769E+0008	5.6615E+0009
VZ mol/Pa	4.9620E+0002	7.9365E+0009	0.0000E+0000	4.6085E+0011
Fugacity Pa	2.7305E-0013	2.7305E-0013	2.7305E-0013	2.7305E-0013
Conc mol/m3	1.1015E-0016	6.6678E-0006	8.4014E-0005	1.5459E-0003
Conc g/m3	2.9741E-0014	1.8003E-0003	2.2684E-0002	4.1738E-0001
Conc ug/g	2.5089E-0011	1.8003E-0003	1.5123E-0002	2.7825E-0001
Amount mol	1.3549E-0010	2.1670E-0003	0.0000E+0000	1.2583E-0001
Amount %	0.000000	1.692993	0.000000	98.307007

4. File 17tre2b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total excretion 17a trenbolone
B:\17tre2b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	1.10000000000000E+0003
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	3.30000000000000E+0001
Sediment/water partition coefficient	6.07200000000000E+0002

Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	1.05356506790590E-0013
Total of VZ products:	1.21492258901879E+0012

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	3.2500E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	3.6800E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	8.0586E+0008	1.4828E+0010
VZ mol/Pa	4.9620E+0002	7.9365E+0009	0.0000E+0000	1.2070E+0012
Fugacity Pa	1.0536E-0013	1.0536E-0013	1.0536E-0013	1.0536E-0013
Conc mol/m3	4.2503E-0017	2.5728E-0006	8.4903E-0005	1.5622E-0003
Conc g/m3	1.1476E-0014	6.9466E-0004	2.2924E-0002	4.2180E-0001
Conc ug/g	9.6808E-0012	6.9466E-0004	1.5282E-0002	2.8120E-0001
Amount mol	5.2278E-0011	8.3616E-0004	0.0000E+0000	1.2716E-0001
Amount %	0.000000	0.653252	0.000000	99.346748

5. File 17tre5b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total excretion
B:\17tre5b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	4.20000000000000E+0002
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	1.26000000000000E+0001
Sediment/water partition coefficient	1.26000000000000E+0002
Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	4.80598546746356E-0013
Total of VZ products:	2.66334554830758E+0011

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	6.5000E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	2.0000E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	3.0769E+0008	3.0769E+0009
VZ mol/Pa	4.9620E+0002	1.5873E+0010	0.0000E+0000	2.5046E+0011
Fugacity Pa	4.8060E-0013	4.8060E-0013	4.8060E-0013	4.8060E-0013
Conc mol/m3	1.9388E-0016	1.1736E-0005	1.4788E-0004	1.4788E-0003
Conc g/m3	5.2348E-0014	3.1688E-0003	3.9927E-0002	3.9927E-0001
Conc ug/g	4.4160E-0011	3.1688E-0003	2.6618E-0002	2.6618E-0001
Amount mol	2.3847E-0010	7.6285E-0003	0.0000E+0000	1.2037E-0001
Amount %	0.000000	5.959803	0.000000	94.040196

6. File 17tre6b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total excretion
B:\17tre6b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	1.10000000000000E+0003
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	3.30000000000000E+0001
Sediment/water partition coefficient	3.30000000000000E+0002
Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	1.90520500004678E-0013
Total of VZ products:	6.71843712339916E+0011

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	6.5000E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	2.0000E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	8.0586E+0008	8.0586E+0009
VZ mol/Pa	4.9620E+0002	1.5873E+0010	0.0000E+0000	6.5597E+0011
Fugacity Pa	1.9052E-0013	1.9052E-0013	1.9052E-0013	1.9052E-0013
Conc mol/m3	7.6859E-0017	4.6525E-0006	1.5353E-0004	1.5353E-0003
Conc g/m3	2.0752E-0014	1.2562E-0003	4.1454E-0002	4.1454E-0001
Conc ug/g	1.7506E-0011	1.2562E-0003	2.7636E-0002	2.7636E-0001
Amount mol	9.4537E-0011	3.0241E-0003	0.0000E+0000	1.2498E-0001
Amount %	0.000000	2.362605	0.000000	97.637395

7. File 17tre7b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total excretion
B:\17tre7b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	4.20000000000000E+0002
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	1.26000000000000E+0001
Sediment/water partition coefficient	2.31840000000000E+0002

Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	2.68500160771448E-0013
Total of VZ products:	4.76722247138451E+0011

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	6.5000E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	3.6800E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	3.0769E+0008	5.6615E+0009
VZ mol/Pa	4.9620E+0002	1.5873E+0010	0.0000E+0000	4.6085E+0011
Fugacity Pa	2.6850E-0013	2.6850E-0013	2.6850E-0013	2.6850E-0013
Conc mol/m3	1.0832E-0016	6.5568E-0006	8.2615E-0005	1.5201E-0003
Conc g/m3	2.9246E-0014	1.7703E-0003	2.2306E-0002	4.1043E-0001
Conc ug/g	2.4671E-0011	1.7703E-0003	1.4871E-0002	2.7362E-0001
Amount mol	1.3323E-0010	4.2619E-0003	0.0000E+0000	1.2374E-0001
Amount %	0.000000	3.329615	0.000000	96.670385

8. File 17tre8b.dat

Program Mlev1a.pas: Four compartment fugacity level 1 calculation
correction for total excretion
B:\17tre8b.dat

Properties of trenbolone

Temperature in degrees C	25.00
Molecular mass in g/mol	270.000
Vapor pressure in pascals	5.46000000000000E-0008
Solubility in g/m3	3.60000000000000E+0002
Solubility in mol/m3	1.33333333333333E+0000
Henrys Law constant in Pa.m3/mol	4.09500000000000E-0008
Log octanol/water partition coefficient	2.70000000000000E+0000
Octanol/water partition coefficient	5.01187233627273E+0002
Organic carbon/water partition coefficient	1.10000000000000E+0003
Air/water partition coefficient	1.65199630743526E-0011
Soil/water partition coefficient	3.30000000000000E+0001
Sediment/water partition coefficient	6.07200000000000E+0002

Number of moles of chemical:	1.2800E-0001
Fugacity in Pa:	1.04672729931598E-0013
Total of VZ products:	1.22285909695530E+0012

Phase properties and compositions

Phase	Air	Water	Soil	Sediment
Volume m3	1.2300E+0006	6.5000E+0002	0.0000E+0000	8.1400E+0001
Density kg/m3	1.1854E+0000	1.0000E+0003	1.5000E+0003	1.5000E+0003
Fraction org C	0.0000E+0000	0.0000E+0000	2.0000E-0002	3.6800E-0001
Z mol/m3.Pa	4.0342E-0004	2.4420E+0007	8.0586E+0008	1.4828E+0010
VZ mol/Pa	4.9620E+0002	1.5873E+0010	0.0000E+0000	1.2070E+0012
Fugacity Pa	1.0467E-0013	1.0467E-0013	1.0467E-0013	1.0467E-0013
Conc mol/m3	4.2227E-0017	2.5561E-0006	8.4352E-0005	1.5521E-0003
Conc g/m3	1.1401E-0014	6.9015E-0004	2.2775E-0002	4.1906E-0001
Conc ug/g	9.6180E-0012	6.9015E-0004	1.5183E-0002	2.7937E-0001
Amount mol	5.1939E-0011	1.6615E-0003	0.0000E+0000	1.2634E-0001
Amount %	0.000000	1.298025	0.000000	98.701975

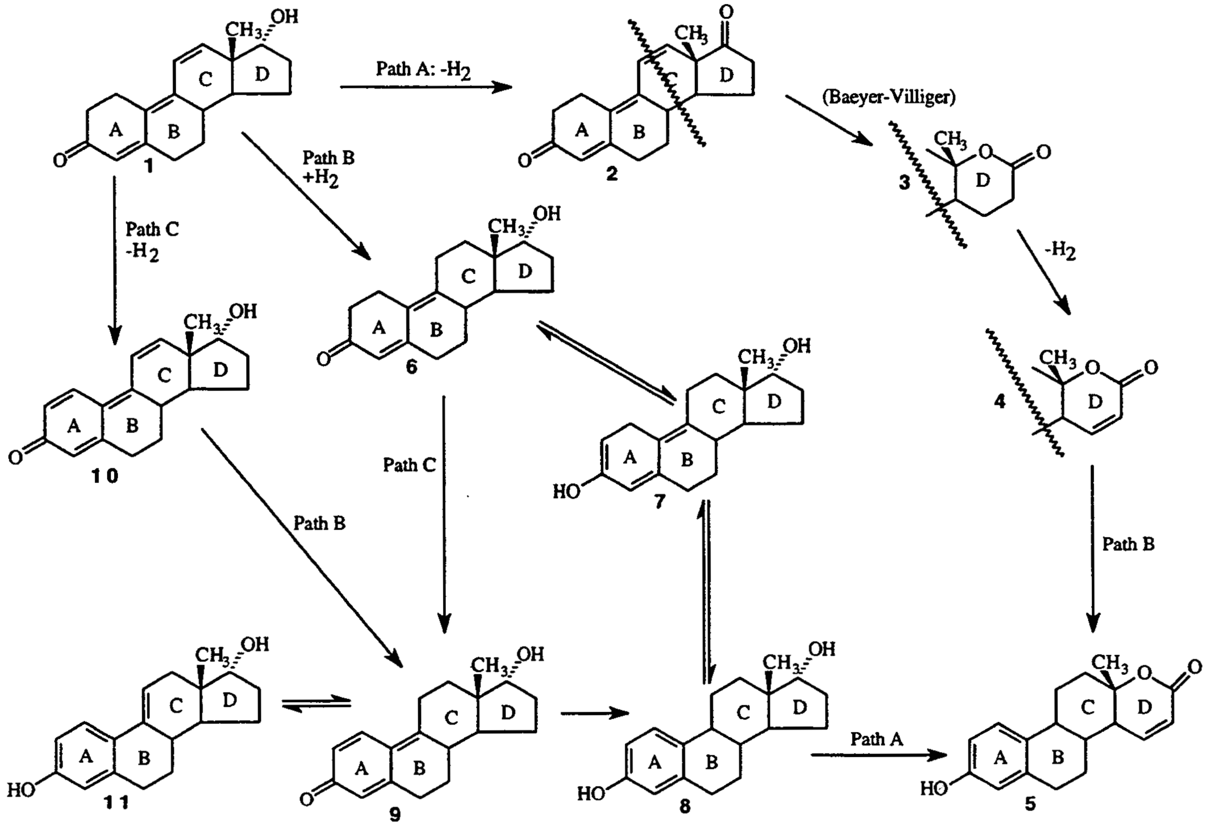
APPENDIX 7

Expanded Summary: Possible Microbial Transformations of
17α-Trenbolone (17α-hydroxyestra-4,9,11-trien-3-one)

Syntex Document # RS-95921 CH 0288

Although no literature exists on the biotransformation of either 17α- or 17β- trenbolone, the basic structure possesses several functional groups that are well known to undergo bioconversion reactions. The major and likely dominant reactions are outlined in the attached Figure. The approach taken to develop this prospective view was based on 25 years of experience in conducting microbial and enzymatic transformations of natural and synthetic compounds, including steroids.

Proposed Metabolic Biotransformations for 17α-Trenbolone



APPENDIX 8

Expanded Summary: Biodegradation of 17α-Trenbolone (17α-hydroxyestra-4,9,11-trien-3-one) by Organisms Extracted from Soil

Syntex Document # RS-95921 CH 0291

The potential for biotransformation of 17α-trenbolone was evaluated in a preliminary study in which a water extract of a field soil was used as a source of organisms. The concentration of 17α-trenbolone in the suspension could be followed by reverse phase HPLC. Correction for adsorption to clay, as well as for day to day variation in chromatography was made by sampling a solution of test chemical in a flask that was subjected to the same conditions as the biodegradation flask. The soil extract contained from 3.5 - 4 mg/mL solids, but no chemicals were present that interfered with the measurement of the test chemical or corresponding metabolites.

Over the period of 20 days, 17α-trenbolone disappeared, and a metabolite formed that was tentatively identified as estra-4,9,11-trien-3,17-dione. The concentration of the metabolite peaked at day 8, then declined steadily to day 20, as shown in the table below. The conversion of 17α-trenbolone to estra-4,9,11-trien-3,17-dione was not quantitative, as other products were formed in small, but measurable amounts.

Area Percentage* of 17α-Trenbolone and Major Biotransformation Products Formed in a Suspension of Field Soil Organisms

Day	Hours	%Parent (R.T. 4.8 min)	%Product 1 (R.T. 6.2 min)	%Product(s) (R.T. 3.2 min)
0	1	91.8	0	0
1	23	81.2	3.1	0
1.5	28	81.5	4.0	0
2	47	77.3	7.9	0
3	71	73.3	14.1	1.9
6	143	45.9	46.8	1.8
8	171	6.6	86.8	1.8
11	267	0	85.3	1.9
14	335	0	81.1	2.3
20	479	0	66.5	2.5

* Area of peak at time t divided by area of parent peak in control sample

APPENDIX 9

Expanded Summary: Estimation of the Maximum Rate of Photolysis of Trenbolone Acetate and 17α-Hydroxy Trenbolone [17α-Trenbolone] in Aqueous Buffer

Syntex Document # RS-95921 CH 0286

The maximum rate of photolysis of trenbolone acetate and principal metabolite, 17α-trenbolone was estimated in accordance with the guidelines in the Environmental Assessment Technical Assistance Handbook (1987). This estimate is based on the ultraviolet-visible absorption spectrum, and assumes a unit quantum efficiency.

The Summer, Fall, and Winter environmental half-lives for trenbolone acetate at a solution pH of 7 were 5.1 sec, 10.2 sec, and 16.6 sec, respectively. The Corresponding half-lives for 17α-trenbolone were 4.8 sec, 9.6 sec, and 15.5 sec. The half-lives for the two chemicals are almost the same owing to very similar ultraviolet spectra.

APPENDIX 10

Expanded Summary: Preliminary Aqueous Photolysis of 17α-Trenbolone

Syntex Document # RS-95921 CH 0290

The purpose of this study was to evaluate the extent of photolysis during a one day exposure to sunlight under winter conditions. The work was conducted in three parts on three separate days, with each part corresponding to an exposure at a designated pH. For photolysis at a pH of 5, a 1.78 x 10^{-5} M 17α-trenbolone solution was prepared in a 0.1 M ammonium phosphate buffer. For photolysis at pH 7, a 2.89 x 10^{-5} M 17α-trenbolone solution was prepared in 0.1 M sodium phosphate buffer. For photolysis at pH 9, a 4.59 x 10^{-5} M solution of 17α-trenbolone solution was prepared in 0.1 M sodium borate buffer. In each study, both foil wrapped and unwrapped quartz tubes containing trenbolone acetate solution were exposed to sunlight. At 30-45 minute intervals, one set of tubes consisting of two exposed and one wrapped tube were sampled for analysis by HPLC. As the control tubes indicated negligible nonphotolytic reactions, the photolytic rate constant was calculated from the best fit of ln (C_0/C_t) versus exposure time, and the half-life calculated from the rate constant.

The test chemical was found to undergo extensive photolysis in a one day period, with half-lives at pH 5, 7, and 9 of 12.6, 3.1, and 3.3 hours, respectively. The longer half-life at pH 5 was due to foggy, cloudy weather conditions.

A ρ-nitroanisole/pyridine actinometer was used on the same day as the study at pH 7 to compensate for variation in the solar radiation during the exposure period. The actinometer samples were exposed and sampled in the same manner as the 17α-trenbolone samples. The corresponding Summer, Fall, and Winter photolysis half-lives are 0.169, 0.355, and 0.608 days, respectively.

APPENDIX 11

Expanded Summary: Microtox® Toxicity Evaluation of Photolyzed Solutions of 17α-Trenbolone

Syntex Document # RS-95921 CH 0292

The purpose of this study was to evaluate the extent of photolysis of 17α-trenbolone during a one day exposure to sunlight under winter conditions in a pH 8.1 sodium bicarbonate buffer. The toxicity of the photolyzed solutions was also followed by Microtox® toxicity assay[1-3] to determine how the toxicity of the 17α-trenbolone photolysis solution changed as 17α-trenbolone was photodegraded.

A 8.73×10^{-5} M 17α-trenbolone solution was prepared in a 0.005 M sodium bicarbonate buffer at pH 8.1. Quartz tubes containing the 17α-trenbolone solution and control tubes, containing 17α-trenbolone solution but wrapped in aluminum foil, were exposed to sunlight. At thirty to forty–five minute intervals, one set of tubes, consisting of two tubes and a control tube, was removed. The concentration of 17α-trenbolone was then determined by high performance liquid chromatography (HPLC) for each tube. As the control tubes indicated negligible nonphotolytic reactions, the photolytic rate constant was calculated by plotting $Ln(C_0/C_t)$ versus time, while the photolytic half-life was calculated from the calculated rate constant. Microtox® toxicity assays were conducted on the photolysis solution in the quartz tubes and the %Light Decrease (a measure of toxicity) was determined for each tube. The decrease in the concentration of 17α-trenbolone was compared to the %Decrease in Toxicity.

17α-Trenbolone undergoes extensive photolysis in a one day period at pH 8.1, with only 10% of the initial 17α-trenbolone remaining after 5.5 hours of sunlight exposure. The photolytic half-life of 17α-trenbolone at pH 8.1 was calculated to be 1.9 hours. Microtox® analysis showed that the toxicity of the exposed 17α-trenbolone solutions decreased approximately 90% by the end of 5.5 hours. The decrease in solution toxicity was proportional to the % decrease in 17α-trenbolone, indicating that any photodegradation product(s) formed were much less toxic than 17α-trenbolone.

References:

1. Bulich, A.A.; Greene, M.W.; Isenberg, D.L. In *Aquatic Toxicology and Hazard Assessment: Fourth Conference*; Branson, D.R.; Dickson, K.L., Eds.; ASTM Spec. Tech. Publ. 737; American Society for Testing and Materials: Philadelphia, PA, 1981; pp 338-347.
2. Blum, D.J.W.; Speece, R.E., *Environ. Sci. Technol.*, **1990**, *24*, (3), pp 284-293.
3. Ribo, J.M.; Kaiser, K.L.E., *Chemosphere*, **1983**, *12*, (11/12), pp 1421-1442.