ENVIRONMENTAL ASSESSMENT

FOR

ZERANOL

SECTION 1.	DATE:	August 19, 1994
SECTION 2.	NAME OF APPLICANT/PETITIONER:	Mallinckrodt Veterinary, Inc.
SECTION 3.	ADDRESS:	421 E. Hawley Street Mundelein, IL 60060

SECTION 4. DESCRIPTION OF THE PROPOSED ACTION:

The applicant proposes to package for over the counter use a 72 mg zeranol implant to be marketed under the trade name of RALGRO® MAGNUM. The 72 mg implants, as well as the currently marketed 36 mg implants with the trade name RALGRO®, will be packaged at the Mallinckrodt Veterinary, Inc. production facility in Terre Haute, IN.

The presently marketed RALGRO® implant consists of three (3) 12 mg pellets and is labeled for increased rate of weight gain and improvements in feed conversion in weaned beef calves, growing beef cattle, feedlot steers and feedlot heifers. RALGRO® is approved for use in suckling beef calves, including those heifer calves intended for breeding purposes, for the claim of increased rate of weight gain. RALGRO® MAGNUM implants will consist of six (6) 12 mg pellets and will be labeled for increased rate of weight gain in feedlot steers.

The active ingredient, zeranol, will be manufactured by a fermentation process already in use at the Mallinckrodt Veterinary, Inc. facility in Terre Haute, IN.

Based upon the proposed action, zeranol could potentially be introduced into the following environments:

a. The environment adjacent to the Terre Haute, IN, manufacturing facility.

b. Agricultural lands where waste products from cattle feedlots are used as fertilizers.

c. Aquatic systems where run-off is collected from sites receiving waste products from cattle feed lots.

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

Chemical Name: (3S,7R)-3, 4, 5, 6, 7, 8, 9, 10, 11, 12-decahydro-7, 14, 16-trihydroxy-3-methyl-1H-2-benzoxacyclotetradecin-1-one

CAS Registry Number: 26538-44-3

Molecular Formula: $C_{18}H_{26}O_5$

Molecular Weight: 322.40

Melting Point: 181-185°C

pKa Values: 8.44 and 11.42 with 1% methanol as co-solvent (Appendix 1)

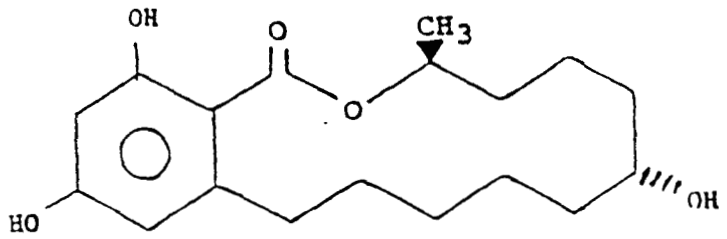
Aqueous Solubility: (Appendix 5)
4.13 µg/mL at pH 5.0
5.14 µg/mL at pH 7.0
27.8 µg/mL at pH 9.0

Vapor Pressure: 3.9×10^{-9} torr (Appendix 2)

N-octanol/Water Partition Coefficient: (Appendix 10)
Log P_{ow} 3.13 at pH 5.0 & 7.0
Log P_{ow} 3.47 at pH 9.0

Ultraviolet Spectrum: UV maxima at 218, 265, 304 nm

Structural Formula:



SECTION 6. INTRODUCTION OF THE SUBSTANCES INTO THE ENVIRONMENT:

Zeranol is prepared commercially from zearalenone which is elaborated as a natural product in submerged cultures of *Gibberella zeae*. Zearalenone is modified by Raney nickel reduction of the 7-ketone. Zeranol is recrystallized to 90-99% purity and forms the basis of the commercial formulation to be known as RALGRO® MAGNUM. This process and recovery method utilizes equipment, procedures and ingredients typical in the pharmaceutical industry. The table below lists the chemicals, in addition to zeranol, that may be used in the manufacture of RALGRO® MAGNUM.

TABLE I

Chemicals Used

3A Alcohol	Char KB
Boric Acid	Ferrous Sulfate
t-Butyl Alcohol	Filteraid
Cerelose	Manganese Sulfate
Lactose	Magnesium Sulfate
Methanol	Magnesium Stearate
Urea	Nickel
Water	Potassium Phosphate
FD&C Yellow #5	Zinc Sulfate

No dangerous air emissions are expected from the manufacture of zeranol. Emissions are regulated under the Clean Air Act, Indiana regulation 326 IAC 2, and administered by the Vigo County Air Pollution Office. The existing fermenters and associated equipment are included in Mallinckrodt's air operating permits numbers 04-2834-01-90 through 04-2834-05-90. All phases of manufacture are equipped with appropriate controls as required by Federal, State and local emission requirements. Construction and air permits have been obtained from the Vigo County Air Pollution Control Office as necessary.

Measurable concentrations of zeranol are not expected in the atmosphere due to zeranol's low vapor pressure (3.9×10^{-9} torr) (Appendix 2). The vapor pressure of zeranol was determined by comparing the gas chromatographic retention time of zeranol to standards of known vapor pressure. The retention times at various column temperatures were then related to vapor pressure using a method described by Kim *et al.* The uncorrected vapor pressure was found to be 1.4×10^{-7} torr at 298 K. When the vapor pressure is corrected for the melting point of zeranol a vapor pressure of 3.9×10^{-9} torr is obtained. This confirms that zeranol will not pose an environmental risk due to volatilization.

SECTION 6. (cont.)

Sanitary disposal will be to the Terre Haute Wastewater Treatment Plant. Non-contact cooling water will discharge to the Wabash River. Monitoring of the discharges is required under the Clean Water Act and conducted in compliance with Mallinckrodt Veterinary's NPDES permit, number IN 0003328. Process wastewater will be treated in an activated sludge treatment plant owned and operated by Mallinckrodt. The wastewater treatment plant will discharge to the Wabash River. Monitoring of the discharges is required under the Clean Water Act and conducted in compliance with Mallinckrodt's NPDES permit, number IN 0003328. Mallinckrodt Veterinary, Inc. currently discharges process wastewater to the Terre Haute Wastewater Treatment Plant under permit 1083.

Solid wastes are regulated under the Resource Conservation and Recovery Act and Indiana regulations 329 IAC 2 and 329 IAC 3. All solid wastes will be disposed at appropriately licensed solid waste disposal facilities. Disposal permits as necessary will be obtained from the Indiana Department of Environmental Management. Solid waste consists primarily of sludge from the wastewater treatment plant operation, rejected process materials and filter cake from recovery operations. Representative samples of the sludge biomass were assayed for heavy metals following the EP (Environmental Protection) Toxicity procedures. All metals were below detection limits. Based on the above information any discharge will comply with appropriate statutes, regulations and permits.

Zeranol will be introduced into the environment primarily through the use of cattle waste as fertilizer. Under the current NADA permits there are approximately 72.75 million implantable cattle (feedlot and pasture) in the U.S. This represents a potential zeranol exposure of 1589 kg at the current market share of approximately 60%. If this NADA is accepted for feedlot steers, the potential exposure will increase by a maximum of 25 % (394 kg) to approximately 1983 kg of zeranol, assuming the current market share of 60 % is maintained. This increase will result from implanting feedlot steers with 72 mg (RALGRO® MAGNUM) instead of 36 mg zeranol (RALGRO®). All controls as outlined in the referenced EA will be maintained.

A worst-case estimate of the concentration of zeranol in feces can be made by assuming that 100% of the zeranol implant is excreted in one day. Feedlot cattle excrete 6% of their body

SECTION 6. (cont.)

weight per day as manure. Therefore, a 1000 lb animal would excrete 60 lbs of manure. This would give a zeranol concentration of 2.6 mg/kg in the feces per 1000 lbs of animal weight (72 mg/(60 lbs X 0.454 kg/lb).

The concentration of zeranol in the environment due to the practice of using manure as fertilizer can be estimated. The rate of excretion of zeranol can be estimated by assuming that 100% of the administered zeranol is excreted in the feces at a constant rate over the lifetime of the implant (120 days). Using this assumption zeranol would be excreted in the manure at a constant rate of 0.60 mg/day (72 mg/120 days). Manure output from beef cattle may be estimated at 6% of the body weight per day. Therefore 72 mg of zeranol would be excreted in 60 lbs of manure per 1000 lbs of animal weight per day for 120 days for a concentration of 0.02 mg/kg (72 mg/(60 lbs X 120 days X 0.454 kg/lb).

Zeranol degrades in feces with a half-life of eight weeks. The concentration of zeranol in feces can be estimated by summing the equation $1.09e^{-0.012}$ for days 1 thru 120. If you assume that zeranol is excreted at a constant rate over the lifetime of the implant, 0.60 mg will be excreted per day. Using the above expression, 38.5 mg of zeranol will be present in the accumulated manure at day 120. On average cattle are in a feedlot for 140 days prior to slaughter. From day 120 to day 140 no zeranol is added to the manure. The 38.5 mg of zeranol present in the manure will continue to degrade. The amount of zeranol present at the end of 140 days can be estimated by using the following equation:

$$\text{Amount of zeranol} = \text{Zeranol in Manure} \times e^{-0.012t} \quad (1)$$

where t is the time in days.

Using the above equation, approximately 24.0 mg of zeranol will be present in the manure. $(50.5 \text{ mg} \times e^{-0.012 * 20})$.

It can be estimated that feedlot cattle produce 60 lbs of manure per day. Therefore the total amount of manure produced per cow will be 8400 lbs (60 lbs X 140 days). The concentration of zeranol in the manure will be 0.006 mg/kg from the following equation:

$$\text{Zeranol Conc.} = (24.0 \text{ mg}/(8400 \text{ lb} \times 0.454 \text{ kg/lb})$$

It is common practice to clean pens in a feedlot when the pens are empty. Large feedlots will often store manure prior to application onto farmland. The concentration of zeranol will decrease by 50% every two months. Because manure is stored before application to the field, the actual concentration of zeranol applied will be less than 0.006 mg/kg.

SECTION 6. (cont.)

A typical application rate for spreading manure onto agricultural land is 13,608 kg of manure per acre of land (Thompson, O'Mary, 1983). The weight of one acre of soil incorporating zeranol to a depth of six inches is 909,000 kg. The concentration of zeranol material in soil under these conditions would be:

$$\frac{0.006 \text{ mg zeranol}}{\text{kg manure}} \times \frac{13{,}608 \text{ kg manure}}{\text{acre}} \times \frac{1 \text{ acre}}{909{,}000 \text{ kg}} = 0.90 \times 10^{-4} \text{ mg/kg soil}$$

Typically manure is spread onto the field annually. The amount of zeranol remaining in the field 360 days after application can be estimated from equation 1 to be 0.9×10^{-6} mg/kg or approximately 1 ppt.

The amount of zeranol that could enter the freshwater estruarine and marine ecosystems can also be estimated. Zeranol could enter the marine ecosystems either from feedlot run-off or from field run-off. An estimate of zeranol introduction into the aquatic ecosystem from a feedlot containing all large animals (approximately 1000 lbs) implanted with 72 mg zeranol can be made. Pen area may be estimated at 200 square feet per head (0.0046 acre per head). Assume further that 50.5 of zeranol is present in the manure and contained in run-off water equivalent to a two inch depth in each animals area. Each acre of pen area would contain 5,489 mg of zeranol which would be carried off in two acre-inch of water, equivalent to 205,500 kg of water (102,750 kg per acre-inch). The concentration of zeranol would be 5.3×10^{-2} mg/kg or 0.05 ppm.

Similarly an estimate of zeranol introduction from field run-off can also be made. It is calculated above that zeranol will be present in the soil at a concentration of 0.90×10^{-4} mg/kg after field application of manure. It is assumed that this amount of zeranol is completely desorbed with two inches of water, the concentration of zeranol in the water would be 4.0×10^{-4} mg/kg. This figure is arrived at from the following calculation:

$$\frac{0.90 \times 10^{-4} \text{ mg zeranol}}{\text{kg soil}} \times \frac{9.09 \times 10^{5} \text{ kg soil}}{\text{acre soil}} \times \frac{\text{acre soil}}{2 \text{ acre inch } H_2O} \times \frac{\text{acre inch } H_2O}{102{,}750 \text{ kg}}$$

The scenario above assumes that 100% of the zeranol is contained in the run-off water and each case is mutually exclusive. Adsorption/desorption tests have shown 44% to 58% of the applied zeranol is adsorbed on soil. If it is assumed that 50% of the zeranol binds to the soil, the zeranol concentration present in the run-off is reduced to 2.0×10^{-4} mg/kg. This figure is worst case because zeranol will degrade in the soil and the actual level of zeranol present may be far less than 0.90×10^{-4} mg/kg. This potential level of zeranol does not pose an environmental risk.

SECTION 6. (cont.)

OCCUPATIONAL EXPOSURE EFFECTS FROM COMMERCIAL PREPARATION OF ZERANOL.

Zeranol is a chemical with low estrogenic activity in mammalian species. The effects of zeranol can be attributed to its estrogenic activity. Occupational exposure would be expected to occur in the pelletizing and packaging areas of the plant, and from accidental self-implantation by users. Tyvek® clothing, gloves and respiratory protection (NIOSH/MSHA approved respirator for particulate matter) are recommended for plant personnel. Air monitoring and maintenance of appropriate engineering (ventilation) controls are routinely performed. Label warnings indicate care should be exercised to prevent self-implantation. Appropriate covering of exposed skin and open wounds or cuts is also recommended on the labels.

Review of the toxicity data (Baldwin, 1983; Hidy, 1977) on zeranol indicated that the toxicologic effects of concern for zeranol in the work place would be from its action on the endocrine system (estrogenic activity). Zeranol has low toxicity when administered as a single bolus dose, either orally in the rat and mouse (exceeding 40 g/kg) or intraperitonealy in the rat (female 10,900 mg/kg, male 8,900 mg/kg) and mouse (female only 4,400 mg/kg). Subchronic safety studies and reproductive studies have indicated that the effects observed (decrease in growth rate; endometrial hyperplasia; reduced organ-to-body ratio for testes, ventral prostate, and thymus in males and ovaries in females; increased organ-to-body ratio for adrenals in both sexes and uteri in females) could be attributed to the weak estrogenic activity of zeranol. Pharmacologic tests have demonstrated that zeranol possesses weak estrogenic activity which is between 100 and 1000-fold lower than that of 17 β-estradiol. Multiple long-term studies in mice, rats, dogs and monkeys have shown no evidence of tumorigenicity. Additional studies have indicated no evidence of genotoxicity or teratogenicity for zeranol. Skin absorption of zeranol has been reported; however, the efficiency of absorption is unknown.

Dust production from the manufacturing process may be a concern. Zeranol is not a volatile chemical, so inhalation would be via airborne particulate matter (dust). Use of respiratory protection and ventilation controls during some manufacturing processes reduce the potential for exposure to dust, particularly when this route of exposure is likely.

Review of the published literature for reports on possible effects of occupational exposure to zeranol identified a single paper (Aw, et al., 1989). The investigators reported the effects of occupational exposure to zeranol in a small pelletizing plant. There were 11 exposed and 14 non-exposed workers. Air levels in the production area ranged from 0.683 to 4.000 mg/m^3 the year

SECTION 6. (cont.)

prior to the study and 0.086 to 1.554 mg/m^3 during the study. Data obtained by questionnaire indicated that more breast symptoms were alleged by male and female plant workers compared with non-exposed subjects, although the difference was not statistically significant. Clinical assessment showed no cases of gynecomastia in any of the male participants. Blood samples analyzed by high performance liquid chromatography did not indicate the presence of zeranol, its precursor zearalenone, or its metabolites at levels above the limits of detection (12.2 µg/L for zeranol, 17.1 µg/L for zearalenone, 18.8 µg/L for zearalanone, and 11.5 µg/L for taleranol). Serum levels of follicle stimulating hormone, luteinizing hormone, prolactin and estradiol showed no significant differences between exposed and nonexposed subjects. The data presented in this paper indicate that engineering controls, and personal protection (clothing, gloves and respiratory protection) were adequate to prevent physiological effects of zeranol.

Occupational Safety and Health Administration has not set a Permissible Exposure Limit (PEL) for zeranol, its precursors, or similar compounds. There is a report by Aw, *et al.*, (1985) in the literature suggesting an occupational exposure limit for zeranol should be between 0.05 and 0.30 milligram per cubic meter (mg/m^3) as an 8 hr time-weighted-average (TWA). Based on review of the published literature, several years ago Mallinckrodt Veterinary established an internal standard of 0.1 mg/m^3 as an 8 hr TWA, and has implemented a routine monitoring program for areas of potential exposure in the manufacturing plant.

The primary areas of occupational exposure to zeranol have been identified as the manufacturing (pelletizing and packaging) areas of the plant. Air monitoring for zeranol is routinely performed. Over the past five years, the measured air levels of zeranol have not exceeded Mallinckrodt Veterinary's internal standard of 0.1 mg/m^3 TWA. The values are well below the standard and generally are below the limit of detection (0.001 mg/m^3). Personnel employed in identified exposure areas are required to wear appropriate Tyvek® suits, gloves and respiratory protection which further reduces their potential exposure.

Mallinckrodt Veterinary has provided extensive information on the toxicity data and safety concerns in the product literature. The MSDS and label for zeranol are written to reflect the toxicity data available for the product and its components. In addition, the label includes additional warnings to provide appropriate covering of exposed skin and open wounds or cuts, and to exercise care to prevent accidental self-implantation when administering the material. The latter precaution is no different from that for any implantable material.

SECTION 6. (cont.)

The manufacturing (pelletizing and packaging) areas of the plant have been determined to present the highest potential exposure. As a result of engineering controls, the actual air levels are extremely low, and are consistently below the Mallinckrodt Veterinary internal standard of 0.1 mg/m^3 as an 8 hr TWA. Protective clothing and respiratory protection, required in some areas, further reduce workers' potential exposure. The MSDS and label information adequately reflect the toxicity concerns and provide warnings to prevent ingestion, dermal contact and accidental self-implantation.

SECTION 7. FATE OF THE EMITTED SUBSTANCES IN THE ENVIRONMENT:

The primary manner in which zeranol would be introduced into the environment is through cattle feces collected from cattle feedlot and applied to farmland. Zeranol could enter the aquatic ecosystem by run-off from fields where cattle feces has been applied as fertilizer or by run-off from the feedlot. From the studies described below it is concluded that zeranol will not have an adverse effect on the environment. The half-life of zeranol in manure or soil is less than 90 days.

The potential for zeranol to bioaccumulate or sorb onto organic matter was estimated from the octanol/water partition coefficient (Appendix 10). The partition coefficient was determined at pH 5.0, 7.0 and 9.0. The K_{ow} value (log P_{ow}) ranged from 3.13 to 3.47 as shown in the table below.

TABLE II

Octanol/Water Partition Coefficient

pH	K_{ow}
5.0	3.13
7.0	3.13
9.0	3.47

The partition coefficient was determined by assaying the octanol and water layers of equilibrated solutions for zeranol. The samples were centrifuged and the resulting layers separated and assayed for zeranol. These results indicate the zeranol may bioaccumulate or sorb onto organic matter to a moderate extent. These results also indicate that zeranol would be expected to moderately sorb to soil. This sorption would restrict the movement of zeranol into the aquatic environment.

The sorption of zeranol was determined by performing an adsorption/desorption study on three different soil types (Appendix 3). The preliminary and screening sorption tests were carried out with $CaCl_2$ solution and deionized water. No differences were detected in either solution. In the advanced test only the $CaCl_2$ solution was used. Tables II and III show the percent adsorbed and the adsorption and desorption coefficient for each soil.

SECTION 7. (cont.)

TABLE III

Adsorption Results

Soil	Percent Adsorbed	Kd	Koc	$^1/_n$
Silty Loam (TX)	48	3.92	478	0.929
Loam (CA)	58	6.81	896	0.943
Silty Clay Loam (IL)	44	21.9	809	0.940

TABLE III

Desorption Results

Soil	Percent Adsorbed	Kd	Koc	$^1/_n$
Silty Loam (TX)	52	18.6	2260	1.27
Loam (CA)	42	6.46	845	0.900
Silty Clay Loam (IL)	56	15.5	573	0.640

Distribution absorption coefficients (Kd) range from 3.9 to 21.9 which indicate moderate sorption of zeranol to the three tested soils and subsequent moderate potential environmental mobility. Although zeranol may move through the environment it will be present in low amounts and has been shown to degrade to CO_2 in the environment. As zeranol moved through the environment it would be retarded by the sorption to new soil. This sorption to soil makes it unlikely that any zeranol will reach the aquatic environment.

The potential for zeranol to hydrolyze was determined at various pH values (Appendix 6). Hydrolysis studies were conducted at pH 5, 7, 7.4, 7.8, 8.2, 8.6 and 9 using zeranol of 99+% purity. The test solutions were kept in the dark at 25°C for the duration of the study. Samples were analyzed using HPLC. Initial studies indicated a 17% decline in zeranol concentration at pH 9. Based on this result, a second study was conducted to determine the degradation between pH 7 and 9.

SECTION 7. (cont.)

The results from the second preliminary study gave the rate constants and half-lives given in the table below:

TABLE IV

Hydrolytic Stability of Zeranol

pH	Rate Constant (hr^{-1})	Half-life (days)
5	1.59×10^{-4}	181
7	2.75×10^{-4}	105
7.4	2.93×10^{-4}	98.6
7.8	5.91×10^{-4}	48.9
8.2	8.21×10^{-4}	35.2
8.6	9.38×10^{-4}	30.8
9	1.56×10^{-4}	18.5

The results from the definitive study gave the following values:

TABLE V

Zeranol Concentration, mg/L

pH	Day 0		Day 28	
	Mean	SD	Mean	SD
7	0.957	1.18×10^{-2}	0.933	2.72×10^{-3}
7.4	0.942	1.65×10^{-3}	0.888	2.23×10^{-2}
7.8	0.955	8.11×10^{-3}	0.920	7.30×10^{-3}
8.2	0.972	3.73×10^{-2}	0.906	1.38×10^{-2}
8.6	0.934	2.89×10^{-2}	0.892	6.58×10^{-3}

From the above results it can be concluded that zeranol is hydrolytically stable at pHs 7-8.6. At pH values greater than 8.6 zeranol may hydrolyze.

Aerobic mineralization of zeranol in three soil types has been determined (Appendix 4). Mineralization, as determined by the production of CO_2 ranged from 31.2 to 50.5% of the applied zeranol dose. The soils from the degradation study were assayed for zeranol by HPLC at the conclusion of the study (64 days). HPLC results showed that from 1.2 to 22.7% of the original zeranol remained at the end of 64 days. The results of the aerobic degradation study are summarized in Table VI.

SECTION 7. (cont.)

TABLE VI

Aerobic Degradation Results

Soil Type	Percent Mineralization*	Percent Zeranol Remaining (HPLC)
Silty Clay Loam (Kansas)	31.2	2.4
Clay Loam (Ohio)	35.5	<1.2
Silty Clay Loam (Mississippi)	50.5	22.7

*Determined by cumulative CO_2 evolved.

Analysis of soil by HPLC at day 64 of the study showed less than 23% of the initial dose of zeranol remained in the soil.

It can be concluded from the degradation results that zeranol will degrade in soil to CO_2 and no other byproducts. The time it takes for one half of the zeranol applied to the field to mineralize to CO_2 can be determined from the degradation studies.

The mineralization half-life for each soil is shown in Table VII.

TABLE VII

Mineralization Half-life of Zeranol

Soil Type	Half-life
Silty Clay Loam (KS)	87 days
Clay Loam (OH)	91 days
Silty Clay Loam (MS)	49 days

These studies show that zeranol will mineralize to CO_2 in soil with a half-life of approximately 90 days.

It is industrial practice to spread manure on the field annually. From the table it can be seen that the half-life for zeranol ranges from 49 to 91 days. Therefore if manure is spread on the field every 360 days at a concentration of 0.9×10^{-4} mg zeranol/kg soil, approximately 1 ppt of the applied zeranol will not be mineralized to CO_2. The half-life of zeranol in soil of 91 to 49 days compares well with the preliminary tests that show the half-life of zeranol in manure to be 56 days.

From the adsorption studies it can be concluded that zeranol will moderately bind to soil. Because zeranol will bind to soil it is unlikely that zeranol will be present in field run-off. It is also unlikely that zeranol will migrate in the soil to contaminate rural water systems. It is expected that any zeranol present will bind to the soil and mineralize to CO_2.

SECTION 7. (cont.)

Zeranol is soluble in water at concentrations of less than 5 ppm (Appendix 5). Zeranol is hydrolytically stable for a pH range of 7-8.6 with a half-life of greater than one year at 25°C.

From the above studies it can be concluded that any zeranol present will rapidly degrade in both manure and soil. Zeranol begins degradation immediately, after 56 days approximately 50% of the zeranol present in manure is degraded. This degradation continues after field application of manure. Ninety days after field application 50% of the applied zeranol has mineralized to CO_2. The zeranol that is not degraded would be expected to bind to the soil and not migrate into water systems. Therefore zeranol will not have any effect on the environment.

SECTION 8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

Zeranol may be introduced into the environment in very low concentrations (less than 1.0 ppb). Any zeranol introduced through the use of manure as fertilizer will be mineralized to CO_2 by normal terrestrial fauna.

To ensure that the introduction of zeranol to the environment through the use of manure as fertilizer posed no threat to the environment, studies were conducted to determine the effect of high concentrations (1000 mg/kg, 1 x 10^7 times the expected concentration of zeranol) of zeranol to various environmental systems.

Earthworms (*Lumbricus terrestris*) were exposed to zeranol at nominal concentrations of 1000, 500, 250, 130 and 65 mg/kg for a period of 28 days (Appendix 7). Analysis of duplicate soil samples removed from each control or treatment group established that the zeranol concentration decreased over time within the artificial soil. Soil samples removed at day 14 demonstrated that only the two highest concentrations contained measurable levels of zeranol. At test termination only the highest concentration contained a measurable level of zeranol which averaged 71 mg/kg or 8.2% of the initial dose. At test termination, survival ranged from 93 to 100% among earthworms exposed to zeranol treated soil and the control. There were no significant ($P<0.05$) differences between the survival of control earthworms (95%) and earthworms exposed to the treated soil. Analysis of weight data established no significant difference ($P<0.05$) between the weight of exposed worms compared to the weight of control worms. The LC50 for earthworms exposed to zeranol was empirically estimated to be >870 mg/kg, the highest measured test concentration. Earthworm weight, percentage survival, and burrowing time were no different from the control for any of the comparable groups. Zeranol did not exhibit any toxicity for earthworms in this study at levels over seven million times that expected in the environment.

Because of the rapid degradation of zeranol, studies to determine the effect of zeranol on plant growth were not required. However preliminary studies were conducted to assure that no deleterious effects occurred on seed germination, root elongation and seedling growth when zeranol was present in soil.

To determine the effect of zeranol on seed germination and root elongation, corn, ryegrass, wheat, cucumber, soybean and pinto bean seeds were exposed to a zeranol concentration of 1000 ppm (Appendix 9). Prior to the study seeds were exposed to zeranol concentrations of 1, 10, 100 and 1000 ppm and observed for percent germination or root elongation. No toxic effects were noticed at any of these concentrations and the study was performed at a single nominal concentration of 1000 ppm

SECTION 8. (cont.)

(approximately 1 x 10^7 times the expected concentration. Samples were assayed for zeranol using HPLC methodology. No adverse effects were seen for corn, cucumber, pinto bean, soybean and wheat after exposure for 5 to 6 days to zeranol at 1000 ppm. The no observed effect concentration (NOEC) of zeranol for both seed germination and root elongation for these species was 1000 mg/kg. The lowest observed effect concentration of both parameters was determined to be >1000 mg/kg (approximately 1 x 10^7 times expected concentration in soil). No statistically significant difference was reported for ryegrass seed germination of seeds exposed to 1000 mg/kg zeranol. The ryegrass root length was significantly different from the solvent control but not of the control when exposed to 1000 mg/kg zeranol. The root length is the most variable of all growth parameters. In the range finding study no statistically different effects were seen in the ryegrass seed for zeranol concentrations of 1, 10, 100 and 1000 mg/kg. For these two reasons and the fact that very little zeranol will be present in the soil the difference between the solvent control and treated soil is not significant.

A preliminary study was conducted to determine the effect of zeranol on seedling growth of corn, cucumber, pinto bean, ryegrass, soybean and wheat (Appendix 8). Seedlings were grown in quartz sand containing 1000, 500, 250, 130 or 63 mg zeranol/kg sand. Shoot length was measured on days 1, 3, 5, 7, 14 and 21. On day 21, shoot length, dry shoot weight and dry root weight were measured. Zeranol had no effect ($P<0.05$) on plant survival in any species tested when compared to controls. Except for a depression in root weight in ryegrass, neither shoot length, shoot weight or root weight were affected by zeranol exposure. The NOEC was determined to be 1000 mg/kg (the highest concentration measured) for all species except ryegrass.

From the above data it is concluded that zeranol will not have an adverse effect on the environment. Zeranol may be present in the environment at extremely low levels. The zeranol that is introduced into the environment will be rapidly mineralized to CO_2 by normal terrestrial organisms. Zeranol will have no effect on terrestrial organisms while it is undergoing rapid mineralization. Zeranol has no toxic effect on earthworms or plants at levels approximately ten million times that expected in the environment.

SECTION 9. USE OF RESOURCES AND ENERGY:

Manufacturing zeranol will require an amount of energy similar to that used to produce and package any conventional pharmaceutical product for animals. Disposal of waste washwater and materials from the manufacturing process will not require use of unusual amounts of energy or natural resources. There will be no effects upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

SECTION 10. MITIGATION MEASURES:

As there are no known or expected adverse effects of the proposed action, no mitigation measures will be required.

SECTION 11. ALTERNATIVES TO THE PROPOSED ACTION:

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

SECTION 12. LIST OF PREPARERS:

The following personnel from Mallinckrodt Veterinary, Inc. were responsible for the preparation of this Environmental Assessment:

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8/19/94
Date

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Date

SECTION 13. CERTIFICATION:

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

Diane E. Frazer /sm
Diane E. Frazer
Director, Regulatory Affairs

August 19, 1994
Date

SECTION 14. REFERENCES:

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SECTION 15. APPENDICES:

Test Reports supporting the Environmental Assessment

Appendix Number	Report Title
1.	The Dissociation Constant of Zeranol P-1496
2.	Determination of the Vapor Pressure of Zeranol at 298 K
3.	Determination of the Sorption and Desorption Coefficients of Zeranol from Soil
4.	Zeranol Biodegradation in Soils - Supplemental Study
5.	Determination of Water Solubility of Zeranol
6.	Determination of the pH-dependent Hydrolysis of Zeranol
7.	Subacute Earthworm Toxicity of Zeranol in Soil
8.	Seedling Growth Toxicity of Zeranol
9.	Determination of the Effects of Zeranol on Seed Germination and Root Elongation of Six Plant Species
10.	The Partition Coefficient of Zeranol Between n-Octanol and Aqueous Buffers