I. GENERAL INFORMATION

A. File Number

NADA 138-870

B. Sponsor

The Upjohn Company

C. Proprietary Name

MGA 100/200 Premix, MGA; 500 Liquid Premix, Rumensin,Tylan

D. Established Name

melengestrol acetate, monensin sodium, tylosin phosphate

E. Dosage Form

Feed

F. Dispensing Status

OTC

G. Dosage Regimen

Approval has been granted to feed 0.25 to 0.5 mg melengestrol acetate (MGA) per head per day in combination with 5 to 30 g monensin per ton of air dried complete feed and 8 to 10 g tylosin per ton of air dried complete feed (to provide 90 mg/hd/day) when each additive is provided via separate supplements or a supplement containing MGA is fed in combination with a complete feed containing monensin and tylosin.

The supplement containing melengestrol acetate provided at a rate of 0.5 to 2.0 pounds per head may be top-dressed onto or mixed with the feed containing monensin and tylosin.

This clearance does not provide for the mixing of melengestrol acetate, monensin and tylosin premixes together in a common supplement or in a complete ration offered for sale by the feed manufacturer.

H. Route of Administration

Oral

I. Indication

For increased rate of weight gain, improved feed efficiency, suppression of estrus (heat), and reduced incidence of liver abscesses in heifers fed in confinement.

J. Effect of Supplement

This supplement provides for <changes being approved> (Delete this section for Original approvals)

II. EFFECTIVENESS

The efficacy of melengestrol acetate for increased rate of weight gain, improved feed efficiency and suppression of estrus (heat) in heifers fed in confinement for slaughter is well documented and approved (21 CFR 558.342). Further, the effectiveness of monensin for improved feed efficiency and increased rate of weight gain of feedlot cattle is established and approved (21 CFR 558.311) as is the effectiveness of tylosin for reduction of liver abscesses in the same class of cattle (21 CFR 558.625). For these applications, the efficacy of melengestrol acetate, monensin and tylosin when co-administered was established by conducting a series of field trials under a common protocol. These trials were designed to evaluate the performance of feedlot heifers fed each drug separately and in combination.

A. Pivotal Study

A feedlot heifer study consisting of field trials at nine locations with three replications per location was conducted to generate animal performance data on the combination of melengestrol acetate, monensin and tylosin. Results of each study are reported as well as a summary and statistical analysis of the pooled data from all nine field investigations.

1. **Type of Study:** Clinical (field) trials

2. **Investigator names and addresses by location:**

Trial No. 540-9665-0-JFM-83-002

Steve Rust, Ph.D.
Montana Ag Experiment Station
Route 1, Box 131
Huntley, MT 59037

Trial No. 540-9665-0-JFM-83-003

David Bechtol, D.V.M.
Palo Duro Agri. Division
Box 974
Canyon, TX 79015

Trial No. 540-9665-0-JFM-83-004

Thomas R. Schriemer, B.S.
Unit 9690
The Upjohn Company
Kalamazoo, MI 49001

Trial No. 540-9665-0-JFM-83-005

Steve Rust, Ph.D.
Montana State University
Bozeman, MT 59717

Trial No. 540-9665-0-JFM-83-006

Jack Riley, Ph.D.
Dept. of Animal Science - Industry
Weber Hall KSU
Manhattan, KS 66506

Trial No. 540-9665-0-JFM-83-007

Richard Luther, Ph.D.
Animal and Range Science Dept.
Animal Science Complex Building
Brooklings, SD 57007

Trial No. 540-9665-0-JFM-83-008

Jerry Martin, Ph.D.
Panhandle State University
Box 430
Goodwell, OK 73939
-and-
Donald R. Gill, Ph.D.
005 Animal Science
Oklahoma State University
Stillwater, OK 74074

Trial No. 540-9665-0-83-009*

Elvin Thomas, Ph.D.
Dept. of Animal & Dairy Sciences
Auburn University
Auburn, AL 36830

Trial No. 540-9665-0-JFM-83-010

Wilton Heinemann, Ph.D.
Research & Extension Center
Washington State University
P.O. Box 30
Prosser, WA 99350

Trial No. 540-9665-0-JFM-84-001

Steve Schmidt, Ph.D.
Dept. of Animal and Dairy Sciences
Auburn University
Auburn, AL 36830

*This trial was canceled shortly after it was started for reasons unrelated to the safety and efficacy of the compounds. There were no data generated as a result of this trial.

3. **General design of each field investigation**

   a. Purpose of the field investigation: To evaluate the effect of melengestrol acetate, monensin sodium and tylosin phosphate on the performance of feedlot heifers when fed singly and in combination.

   b. Test animals

      (i) Species: Bovine Number/group: The number of heifers per treatment group varied from location to location, and ranged from 6 to 14 animals per pen (Table 1).

      (ii) Subgroup Identity: Heifers used in the field studies were non-pregnant yearlings and the breeds were typical of those commonly found in commercial feedlots. The average initial weights ranged from 674 to 826 pounds (Table 1).

   c. Control groups

      Groups of heifers fed melengestrol acetate, or monensin or tylosin alone, or monensin and tylosin in combination served as controls. Pairwise, comparisons were made to groups of heifers for the 3 drugs in combination.

   d. Diagnosis: Not applicable.

   e. Dosage form: (1) Medicated feed: Feed was medicated using melengestrol acetate, monensin or tylosin premixes. Final feeds were prepared using intermediate premixes which were either mixed into or top-dressed onto the bulk of the ration.

   f. Route of drug administration: (1) Oral

   g. Dosages: The dosages used in the field study were: melengestrol acetate, 0.5 mg/head/day; monensin, 30 gm/ton of air dried feed; tylosin, 10 gm/ton of air dried feed.

   h. Days on test: The cattle were fed for a minimum of 90 days, the actual day of trial termination being left up to the investigator's judgement as to when the cattle had reached market weight and condition. The days on test by location are listed on Table 1.

Table 1. General Trial Information

Location / Trial No.	Mean Starting		Estrus Detection	
	Number/Pen	Wt/lbs	Days on Test	(Yes/No)
Montana (Huntley) (A)/ 540-9665-0-JFM-83-002	12	790	102	Yes
Texas / 540-9665-0-JFM-83-003	14	707	104	Yes
Michigan / 540-9665-0-JFM-83-004	8	735	97	No
Montana (Bozeman) (B) / 540-9665-0-JFM-83-005	8	826	97	Yes
Kansas / 540-9665-0-JFM-83-006	6	731	92	No
South Dakota / 540-9665-0-JFM-83-007	8	700	126	No
Oklahoma / 540-9665-0-JFM-83-008	8	674	112	Yes
Washington/ 540-9665-0-JFM-93-010	8	773	96	Yes
Alabama / 540-9665-0-JFM-001	8	728	99	No

i. Measurements taken: The following were measured:

(i) Estrus: 2X per day (AM and PM) during days 5 through 47 of the trial. Estrus was measured at five of the nine locations (Table 1).

(ii) Cattle weight: Cattle were weighed at about 28 day intervals.

(iii) Feed consumption: Daily feed records were maintained.

(iv) Daily observations: Individual pens of cattle were observed at least once per day for general activity and physical condition of the cattle.

(v) Liver Evaluation: Individual heifer livers were evaluated for liver abscesses at slaughter.

4. **Results**

Performance Data: The treatment by location means for percent standing estrus, average daily gain, feed efficiency and percent abscessed livers are presented in the following tables. While the study included several treatment groups, data from only those pertinent to the subject approval are included.

Table 2. Treatment by Location Means for Percent Standing Estrus

Treatment	Oklahoma	Montana*	Montana**	Texas	Washington
melengestrol acetate	8.33	16.67	16.67	26.19	8.33
tylosin	41.67	45.83	80.56	30.95	62.50
melengestrol acetate/ tylosin	8.33	16.67	16.67	26.19	8.33
melengestrolacetate/ monensin/tylosin	0.00	20.83	13.89	19.05	0.00

* Huntley, Montana
** Bozeman, Montana

Table 3. Treatment by Location Means for Average Daily Gain

Treatment	Alabama	Oklahoma	Kansas	Montana*	Montana**	S Dakota	Texas	Michigan	Washington
melengestrol acetate	2.90	2.18	3.18	2.46	2.89	3.30	3.10	3.45	2.46
tylosin	2.48	2.08	3.03	2.36	2.77	3.04	2.72	3.14	2.16
melengestrol acetate/ tylosin	2.63	2.39	3.17	2.40	2.93	3.19	2.92	3.42	2.52
melengestrol acetate/ monensin/ tylosin	3.01	2.43	2.90	2.73	2.91	3.36	2.72	3.37	2.36

* Bozeman, Montana
** Huntley, Montana

Table 4. Treatment by Location Means for Feed Efficiency

Treatment	Alabama	Oklahoma	Kansas	Montana*	Montana**	S Dakota	Texas	Michigan	Washington
melengestrol acetate	8.30	6.91	8.21	10.06	9.17	6.52	7.28	6.98	8.48
tylosin	8.43	6.81	8.33	10.64	9.35	6.86	7.88	7.41	9.02
melengestrol acetate/ tylosin	8.53	6.25	8.19	10.42	9.03	6.53	7.49	6.85	8.49
melengestrol acetate/ monensin/ tylosin	7.53	5.86	7.90	8.95	8.67	6.37	7.60	6.41	8.40

* Bozeman, Montana ** Huntley, Montana

Table 5. Treatment by Location Means for Percent Abscessed Livers

Treatment	Alabama	Oklahoma	Kansas	Montana*	Montana**	S Dakota	Michigan	Washington
melengestrol acetate	16.67	16.67	0.00	41.67	14.14	8.33	3.33	16.67
tylosin	0.00	4.17	5.56	12.50	11.11	0.00	0.00	8.33
melengestrol acetate/ tylosin	12.50	0.00	0.00	8.33	11.11	4.17	0.00	8.33
melengestrol acetate/ monensin / tylosin	4.17	4.17	5.56	16.67	5.56	8.33	0.00	16.67

* Bozeman, Montana ** Huntley, Montana

The results from the 9 individual field investigations were pooled and analyzed statistically to evaluate effectiveness. Average daily gain (ADG) and feed efficiency (FE) data were pooled from all nine locations (Tables 3, 4), estrus suppression data were pooled from five of the nine locations (Table 2) and liver abscess data were pooled from 8 locations (Table 5).

The least squares treatment means pooled across all locations are presented in Table 6.

Table 6. Least Squares Treatment Means Pooled Across Location

	Variable			
Treatment	**% Standing Estrus*1**	**Feed Efficiency**	**Average Daily Gain**	**% Abscessed Livers**1**
melengestrol acetate	16.21	7.99	2.88	24.76
tylosin	46.35	8.30	2.64	15.51
melengestrol acetate/tylosin	23.83	7.97	2.84	16.19
melengestrol acetate/ monensin/tylosin	19.17	7.52	2.87	16.13

* Data from five of nine locations
** Data from eight of nine locations
1 % Standing Estrus and % Abscessed Livers means are based on Freeman-Tukey Transformation.

Statistical Analysis: A least squares analysis of variance for the transformed liver abscess and performance data from the nine locations, and transformed estrus data from 5 locations was conducted. The results of this analysis are presented on Table 7.

Table 7. The significance levels for the comparisons required for the combinations were:

		Parameter			
Combination	**Comparison**	**Estrus**	**FD**	**ADG**	**Liver Abscess**
MGA + Monensin(M) + T	MGA + T vs MGA + M + T		.00015		
	T vs MGA + M + T	.0000003		.00010	
	MGA vs MGA + M + T				.00752

The combination of melengestrol acetate, monensin and tylosin was justified. In the melengestrol acetate + tylosin vs. melengestrol acetate + monensin + tylosin contrast, the contribution of monensin for increased feed efficiency (P = 0.00015) was demonstrated. In the tylosin vs. melengestrol acetate, tylosin, and monensin contrast, the estrus suppression effect of melengestrol acetate

was demonstrated (P = 0.0000003) as was the average daily gain contribution of melengestrol acetate and monensin (P = 0.00010). The contribution of tylosin for control of liver abscesses (P = 0.00752) was evident in the melengestrol acetate vs. melengestrol acetate, monensin and tylosin contrast (Table 7).

5. **Conclusions**

The data generated by the nine location field study demonstrated that the melengestrol acetate, monensin, tylosin combination is justified for it's effect on estrus suppression, improved feed efficiency, increased average daily gain and reduction of liver abscesses.

6. **Adverse Reactions**

No adverse reactions due to the feeding of melengestrol acetate, monensin, or tylosin, either singly or in combination were reported.

7. **Special Issues**

a. Compliance with combination drug policy:

The effectiveness and safety of melengestrol acetate, monensin, and tylosin when fed singly to feedlot cattle are well documented in their respective approved NADAs. The results of adequately controlled studies were submitted to the FDA in support of these respective NADAs. Such data resulted in the approval of melengestrol acetate as per CFR 558.342, monensin as per CFR 558.355 and tylosin as per CFR 558.625. Data generated in support of combination usage have demonstrated that melengestrol acetate, monensin and tylosin fed together are effective and safe.

The data support compliance with 21 CFR 514.1(b)(8)(v) and CVM's combination drug guidelines (November 9, 1983). The combination is justified as detailed in sections 4, 5 and 6 of this application.

The use of untreated controls was not deemed necessary as a test for general effectiveness because the individual drugs have been previously shown to be effective and already approved at the levels tested. The treatment groups consisting of the individual drugs and the two-way combinations served as the appropriate controls for the respective two-way combination. Thus, these treatment groups essentially served as negative controls with respect to 21 CFR 514.111(a)(5)(ii)(a)(4), an the studies were properly controlled.

To justify the combination, comparisons were made which demonstrated that each drug made a statistically significant contribution to the combination. The three-way combination provides a benefit that cannot be obtained from any of the possible two-way combinations.

A comparison of melengestrol acetate vs melengestrol acetate-monensin-tylosin for transformed percent liver abscesses demonstrates the

contribution of tylosin to the combination. This comparison is based on the the fact that neither melengestrol acetate nor monensin has an effect on liver abscesses. Data in the monensin-tylosin NADA (104-646) verify the assumption that monensin has no effect on liver abscesses and data in NADA (138-995) verify that melengestrol acetate has no effect on liver abscesses.

A comparison of tylosin vs melengestrol acetate-monensin-tylosin addresses melengestrol acetate's contribution to the combination. Since melengestrol acetate has estrus suppression activity and monensin does not, this comparison is a valid measure of the effect of melengestrol acetate in the combination. This assumption is verified by data in NADA 124-309 (melengestrol acetate-monensin).

A comparison of melengestrol acetate-tylosin vs melengestrol acetate-monensin-tylosin addresses monensin's contributions that significantly improved feed efficiency is available with the three-way combination when compared to the two-way, melengestrol acetate-tylosin.

The proposed ranges for each drug are justified when fed in combination since the highest levels proposed were tested in combination for efficiency and human and animal safety.

B. Corroborative studies: N/A

III. TARGET ANIMAL SAFETY

A. Pivotal study

The safety of melengestrol acetate, monensin, and tylosin in the bovine have been documented as evidenced by their respective approvals (21 CFR 558.342 for melengestrol acetate, 21 CFR 558.355 for monensin, and 21 CFR 558.625 for tylosin) as feed additives. The following pivotal study was conducted to establish the target animal safety of these three additives when fed in combination.

1. A 90 day, subchronic study was conducted.

2. Investigator:

   A.D. Hall, Ph.D., D.V.M.
   The Upjohn Company
   Kalamazoo, MI 49001

3. General Study design:

   a. Purpose: The purpose of this study was to evaluate the safety of the melengestrol acetate, monensin and tylosin when fed in combination in the diet of yearling heifers at 1 to 5 times the recommended dosages.

   b. Test animals:

      (i) Species: Bovine Number per Group: 14 control, 7 treated.

      (ii) Breed: Hereford

(iii) Age: Yearling

(iv) Sex: Female (heifers)

(v) Weight: 284 to 378 kg initial weight

c. Dosage form:

(i) Drug premixes were incorporated in the feed through the use of intermediate premixes.

(ii) Formulations were typical of those used in commercial feedlot.

d. Dosage used:

(i) Control: No drugs

(ii) 1X:

0.5 mg melengestrol acetate per head per day
30 g monensin sodium per ton of air dried feed
10 g tylosin phosphate per ton of air dried feed

(iii) 3X:

1.5 mg melengestrol acetate per head per day
90 g monensin sodium per ton of air dried feed
30 g tylosin phosphate per ton of air dried feed

(iv) 5X:

2.5 mg melengestrol acetate per head per day
150 g monensin sodium per ton of air dried feed
50 g tylosin phosphate per ton of air dried feed

e. Routes of administration: Oral

f. Test duration: 90 days

g. Parameters measured:

(i) Clinical observations included twice daily health checks, daily observations, body weight, daily feed consumption.

(ii) Clinical pathology

The following hematology parameters were measured:
Total leukocyte count
Total erythrocyte count
Hemoglobin
Hematocrit
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Platelets

Microscopic Examination: WBC differential

The following serum chemistry parameters were measured:

Aspartate aminotransferase
Alkaline phosphatase
Creatinine
Inorganic Phosphorus
Glucose
Blood Urea Nitrogen
Calcium
Cholesterol
Total Bilirubin
Total Protein
Albumin
Creatinine phosphokinase
Sodium
Potassium
Chloride
Sorbitol dehydrogenase

Urine specimens were collected via paracentesis from the urinary bladder at the time of necropsy. The following parameters were measured:

Specific gravity

Dipstick:
- pH
- Protein
- Glucose
- Ketones
- Bile

Microscopic Examination: Formed elements

(iii) Gross and microscopic pathology

The following is a list of organs and tissues that were examined grossly and had a representative sample fixed in 10% neutral buffered formalin for microscopic evaluation:
Brain
Pituitary
Thyroids
Adrenals
Pancreas
Ovaries
Uterus
Mammary gland
Mediastinal lymph node

Mesenteric lymph node
Lung
Liver
Kidneys
Urinary bladder
Spleen
Gross lesions
Diaphragm
Rumen
Reticulum
Omasum
Abomasum
Duodenum
Jejunum
Ileum
Cecum
Colon
Heart (one section from each atrium and ventricle)
Gall bladder
Aorta (and small arteries)
Bone
Bone Marrow (smear)*
*Bone marrow smears were made at the time of necropsy, air dried and fixed for 30 seconds or more in methanol. Slides were stained with Wrights stain.

The following organs were weighed at necropsy, paired organs weighed together:

Adrenals, liver, kidneys and heart.

(iv)Results

(a) Clinical Observations: No adverse, drug-related clinical signs were observed during this study. Although there were no significant differences in average daily gain (ADG) between treated and control animals at mid-study and at termination, there was a trend toward decreased ADG at the 3X and 5X levels of treatment.

(b) Clinical pathology: Occasional statistically significant changes in several hematologic and serum chemistry parameters were detected, but all values were well within normal acceptable ranges and no dose-related trends were seen.

(c) Gross and microscopic pathology: Several gross and histologic tissue changes were observed, but due to the relative even distribution of the changes in all dose groups including controls, these findings were considered to be incidental rather than drug-related.

h. Conclusions:

Based upon the results of this study, the combination of melengestrol acetate, monensin and tylosin phosphate in the daily feed of yearling heifers appears to be non-toxic when fed for 90 days at up to 5 times the recommended dose.

IV. HUMAN FOOD SAFETY

A. Drugs for use in food animals

1. Toxicity tests

Data regarding toxicity testing in melengestrol acetate, monensin sodium and tylosin phosphate are contained in the approved NADAs for the three above mentioned compounds.

2. Safe concentration of residues

Tolerances for melengestrol acetate, monensin sodium and tylosin phosphate are published in the Code of Federal Regulations. Melengestrol acetate is currently approved under 21 CFR 558.342 for use in heifers at 0.25 to 0.50 mg/head/day with a 48-hour withdrawal period. No residues of melengestrol acetate may be found in uncooked edible tissues of cattle with a method sensitive to 25 ppb (21 CFR 556.380). The tolerance for monensin in edible tissue of cattle under 21 CFR 556.347 is 0.7 ppm with no withdrawal period established for cattle under 21 CFR 558.311. The tolerance for tylosin phosphate has been established at 0.2 ppm (negligible residue) in uncooked fat, muscle, liver and kidney (CFR 556.740). Tylosin phosphate has no withdrawal period established under 21 CFR 558.625.

3. Metabolism and total residue depletion studies

Numerous studies have been conducted relative to the metabolism and depletion of residues of melengestrol acetate, monensin, and tylosin when administered individually to cattle. The results of those studies have been filed under the following submissions:

Melengestrol Acetate	**Monensin Sodium**	**Tylosin Phosphate**
NADA 34-254	NADA 38-878	NADA 12-491
NADA 39-402	NADA 95-735	NADA 104-646
NADA 124-309		

4. Residue depletion noninterference study

The following study was conducted to support the use of the three-way combination of melengestrol acetate, monensin sodium and tylosin phosphate in heifers:

Location: Michigan

Sponsor: The Upjohn Company

Investigators:

L.F. Krzeminski, Ph.D.
The Upjohn Company

P. R. Handy, Ph.D.
Lilly Research Laboratories
Greenfield, IN 46140

Groups of feedlot heifers were fed for 90 days with the drug combinations and treatment levels described below.

Group	**Number of Animals**	**Treatment Level**	**Treatment***
1	14	0	Control
2	7	1X	0.5 mg MGA + 30 g monensin + 10 g tylosin
3	7	3X	1.5 mg MGA + 90 g monensin + 30 g tylosin
4	7	5X	2.5 mg MGA + 150 g monensin + 50 g tylosin

* Melengestrol acetate treatment expressed as mg/head/day, monensin and tylosin expressed as gram/ton air-dried ration.

The animals in each group were slaughtered within 16 hours following their last feeding. Perirenal fat was collected from all four treatment groups for the melengestrol acetate analysis, and liver samples were collected from the group 1 and 2 animals for the assay of monensin and tylosin.

a. Residues of melengestrol acetate

The samples of pererenal fat from each animal were assayed for residues of melengestrol acetate using the official AOAC gas chromatographic method. Analysis of the samples from the group 2 (1X) animals showed that all fat samples were below the 25 ppb tolerance for MGA and five of the seven samples were below the 10 ppb limit of reliable measurement of the assay. The two fat samples that gave positive responses had MGA levels of 12.7 and 13.7 ppb.

Fat samples from groups 3 and 4 (the heifers dosed at 3X and 5X levels) yielded average residue levels of 37.6 and 49.4 ppm, respectively.

b. Residues of monensin

Liver tissue that was collected from the seven control animals and the seven heifers fed the group 2 (1X) ration were assayed for monensin by a method that measures microbiologically active residues of the drug. No positive responses in any of the samples were obtained by this method which has a limit of quantitation of 0.04 ppm.

c. Residues of tylosin

The liver tissue samples from the group 2 (1X) heifers and seven of the control animals were assayed for tylosin by the official assay which measures microbiologically active residues of the drug. No positive responses were obtained for any of the samples by this method which has a limit of quantitation of 0.1 ppm.

5. Assay noninterference data

a. Melengestrol acetate assay

Data were generated using spiked control fat tissue samples to demonstrate that the presence of tylosin and monensin does not interfere with the assay of MGA. The study was also intended to show that MGA is stable in frozen fat tissue over a period of 60 days.

Samples of freshly ground control bovine fat tissue were spiked with MGA, tylosin and monensin at the following levels:

MGA: 0.025
monensin sodium: 5.0 ppm
Tylosin phosphate: 1.0 ppm

The spiked samples were assayed in duplicate for MGA by the official AOAC method after 0, 15, 30, 45, and 60 days of storage at -20°C. The average recoveries are shown below.

	Day of Assay					**Ave.**	**S.D.**
	0	**15**	**30**	**45**	**60**		
Percent Recovery	93	88	109	101	98	98	8.0

b. Monensin assay

The data to demonstrate that the presence of tylosin and MGA does not interfere with the assay of monensin in cattle liver are contained in study S-AAC-84-02 conducted by Elanco Products Company. In that study, samples of liver tissue from control heifers were composited and fortified with 0.06 ppm monensin, 0.2 ppm tylosin and 0.2 ppm MGA. The analysis of those samples by the thin-layer bioautographic method for monensin showed no interference by tylosin and MGA.

The stability of monensin in frozen liver tissue was demonstrated through the use of fortified control samples. Liver tissue from control animals was spiked with monensin at 0.06 ppm, frozen for 26 days, and then assayed for monensin. Recoveries averaged 104% of the theoretical spike.

c. Tylosin assay

Data to demonstrate that the presence of MGA and monensin does not interfere with the assay of tylosin were generated by Elanco Products

Company in study S-AAC-84-02. Samples of liver from control heifers were composited and fortified with 0.2 ppm MGA, 0.2 ppm monensin, and 0.2 ppm tylosin. The samples were assayed for tylosin by the cylinder plate microbiological method using Micrococcus luteus as the test organism. No interference was detected by the presence of monensin and MGA.

The stability of tylosin in frozen liver tissues was demonstrated in study MRC8910 conducted by the Elanco Products Company. Liver tissue from control animals was fortified with 0.2 ppm tylosin and then frozen. Portions of the samples were thawed at intervals up to 56 days and assayed for tylosin. The stability of tylosin through 56 days was indicated by the recoveries in the following table:

Tylosin Storage Stability in Frozen Liver

Storage (days)	Mean Tylosin Recovery (ppm)	Percent Theorectical
0	0.166	83%
14	0.239	114%
28	0.172	86%
56	0.162	81%

Regression analysis of these data demonstrates satisfactory tylosin stability for the period the tissues in the residue depletion study were stored before assay.

The residue depletion and assay noninterference studies presented above demonstrate that the combined feeding of melengestrol acetate, monensin sodium, and tylosin phosphate at their highest approved levels results in tissue residues below the tolerance levels for each of the three drugs at 16 hours of withdrawal. The data also show that each drug in the three-way combination does not interfere with the assays for the others. This work confirms the adequacy of the 48 hour withdrawal time required for the presence of MGA in the combination and demonstrates that the use of these feed additives in combination does not pose a hazard to public health.

6. Regulatory methods

Practical analytical methods of analysis for tissue residues of melengestrol acetate, monensin sodium and tylosin phosphate may be found in the Food Additives Analytical Manual on display in

FDA's Freedom of Information
Public Room (Room 12-A-30)
5600 Fishers Lane
Rockville, MD 20857.

V. AGENCY CONCLUSIONS

These NADAs provide for the combination use of MGA, monensin sodium, and tylosin phosphate at the levels of 0.25 to 0.5 mg/head/day, 5 to 30 g/ton of feed, and 8 to 10 g/ton of feed (to supply 90 mg/head/day), respectively; for increased rate of weight gain, improved feed efficiency, suppression of estrus (heat), and reduced incidence of liver abscesses in heifers fed in confinement for slaughter. Adequate data were submitted which show that the MGA, monensin, tylosin combination is justified for its effects on estrus suppression, improved feed efficiency, increased rate of weight gain, and reduction of liver abscesses. Each drug; MGA, monensin, and tylosin has been shown to make a significant ($P < .05$) contribution to the effectiveness of the combination. The combination, when fed at up to 5X the highest recommended combination approval (0.5 mg MGA/head/day plus 30 g monensin/ton, and 10 g tylosin phosphate/ton for 90 days), did not produce an adverse effect. No changes were made in the approved levels of the compounds or in the target animal and the non-interference of tylosin phosphate, monensin sodium, and MGA with the analytical methods for MGA, monensin and tylosin phosphate, respectively, was demonstrated. Accordingly, approval of theses NADAs is not expected to increase human exposure to drug residues, and therefore did not require a complete re-evaluation of the human safety data in the original applications. For the purposes of human food safety review, these original NADAs have been treated as Category II supplements under the Agency's Supplemental Policy (42 FR 64367). These production drugs are OTC because they do not raise any special safety concerns.

VI. ATTACHMENTS

1. Type C Medicated Feed package label and mixing instructions
2. Type C Medicated Feed Liquid supplement package label and mixing instructions

Copies of these labels may be obtained by writing to the:

Freedom of Information Office
Center for Veterinary Medicine, FDA
500 Standish Place
Rockville, MD 20855

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.