I. GENERAL INFORMATION

A. File Number

NADA 139-913

B. Sponsor

Solvay Veterinary, Inc.
PO Box 7348
Princeton, NJ 08540

C. Proprietary Name

Equron

D. Established Name

hyaluronate sodium

E. Dosage Form(s), Route of Administration and Recommeded Dosage

The dose of Equron in small and medium size joints (fetlock, carpal) is 2 ml (10 mg) administered intra-articularly. In larger joints (hock), the dose is 4 ml (20 mg). Depending on the clinical response and medical judgement of the veterinarian, the treatment may be repeated weekly for a total of four treatments.

Strict aseptic measures should be taken to prepare the site for injection and during the intra-articular administration of Equron. Care should be used while injecting to avoid scratching the cartilage surfaces. Such trauma can result in diffuse, transient swelling lasting 24 to 48 hours, but will have no detrimental effect on the ultimate clinical response. For best results, the horse should be given two days of stall rest before gradually resuming normal physical activity.

F. Dispensing Status

Prescription (Rx)

G. Indication

Equron is indicated for the treatment of joint dysfunction in horses due to noninfectious synovitis associated with equine osteoarthritis.

II. EFFECTIVENESS

A. Pivotal Studies

1. Dose Determination

A dose determination study was conducted between April 30 and June 6, 1985 at the Meadowlands, Racetrack, East Rutherford, NJ under the direction of Dr. Kenneth Seeber, Oak Hollow Farm, Gladstone, NJ. Equron injections were performed by Dr. Seeber and clinical evaluations were performed by his associate Dr. Kevin Skinner. Synovial fluid analyses were done by Chesapeake Biological Laboratories, Inc., Hunt Valley, MD.

a. Study Design

The purpose of this study was to evaluate the clinical and synovial fluid response of racing horses with joint lameness to various dosages of Equron. Clinical cases actively in training or racing demonstrating lameness of the carpal joint were selected by the directing veterinarian. Selection criteria for the clinical cases entered into the study were: male or female, between 2 and 3 years of age, with lameness of a carpal joint demonstrating heat, effusion, and pain upon flexion. Only one joint per animal was treated. Upon acceptance into the study each case was randomly assigned to one of 4 treatment groups: control 0.5 mg, 5 mg, 10 mg, and 20 mg Equron. Each group consisted of 10 cases, prior to treatment each case was graded for lameness, flexion, heat, pain and swelling an a synovial fluid sample obtained for determination of relative viscosity and protein concentration. A single intra-articular injection of the assigned Equron dose was administered and at 5-7 days post-injection clinical evaluations and synovial fluid samplings were repeated. Both the clinical evaluations and the synovial fluid analyses were conducted blind.

b. Results

All forty cases displayed clinical signs of synovial inflammation of the carpal joint prior to treatment. The condition was characterized by reduced range of flexion, pain upon pressure, swelling, heat and lameness. Pretreatment synovial fluid analysis revealed reduced relative viscosity in all cases. The majority of the synovial protein concentrations were within the normal range.

At the post-treatment evaluation period, 9 of the 10 animals in the control group (0.5 mg dose) had shown no change in their clinical lameness with one case showing slight improvement. In the 5 mg group 2 cases remained unchanged, 5 showed minimal improvement and 3 had returned to soundness. All 10 cases in the 10 mg group were judged sound at the post-treatment evaluation. Of the cases in the 20 mg group, 9 were sound after 1 week. The single case in this group which did not achieve soundness was however greatly improved.

Post-treatment synovial fluid analysis of the affected joints showed an increase in the mean relative viscosity with increasing dosage of Equron. Each group exhibited some variation in the magnitude of change between individual animals. Since the pretreatment synovial fluid protein concentrations were generally normal, no change in this parameter as a result of Equron injection would be expected. Protein concentrations remained largely within the normal range with only a few cases showing slight elevations at the post-treatment analysis. Such variations ere random and not related to any specific dose group. Throughout the study all injections were well tolerated. Despite the dependency of efficacy on dose, no exacerbation of the pretreatment condidtion or post-injection reactions were observed in any case.

Table 1A summarizes the clinical results for pre and post-treatment intervals for flexion, and clinical summary score. Table 1B summarizes the

laboratory results of the pre and post-treatment intervals for relative viscosity and protein concentration of the synovial fluid.

TABLE 1A PRE-TREATMENT AND POST-TREATMENT MEANS FOR CARPAL FLEXION AND CLINICAL SUMMARY SCORE

	Dose	N	Mean	Std. Dev	S.E. Mean
Pre-treatment Degrees Flexion*	0.5 mg	10	46.500	20.956	6.6270
	5.0 mg	10	48.000	22.261	7.0396
	10.0 mg	10	49.000	23.428	7.4087
	20.0 mg	10	36.500	25.501	8.0640
Post-treatment Degrees Flexion	0.5 mg	10	47.5000	20.310	6.4226
	5.0 mg	10	70.500	17.709	5.6001
	10.0 mg	10	90.000	0.000	0.0000
	20.0 mg	10	77.500	23.717	7.5000
Pre-treament Clinical Score**	0.5 mg	10	6.500	1.716	0.5426
	5.0 mg	10	6.000	2.309	0.7303
	10.0 mg	10	6.300	2.312	0.7311
	20.0 mg	10	7.800	2.394	0.7572
Post-treatment Clinical Score	0.5 mg	10	6.000	1.826	0.5774
	5.0 mg	10	2.500	1.841	0.5821
	10.0 mg	10	0.000	0.000	0.0000
	20.0 mg	10	1.500	2.461	0.7782

* Flexion was measure in degrees from vertical with 90 considered full flexion.
** Clinical score is the sum of ranking (0-3) for heat, pain, swelling and lameness. (0 = no evidence; 1 = slight; 2 = moderate and 3 = severe). Maximum clinical score = 12 and minimum clinical score = 0.

TABLE 1B PRE-TREATMENT AND POST-TREATMENT MEANS FOR CARPAL RELATIVE VISCOSITY AND PROTEIN

	Dose	N	Mean^{+}	STD. DEV.
Pre-treatment Degrees Flexion*	0.5 mg	10	7.8050	3.4527
	5.0 mg	10	10.9900	3.8911
	10.0 mg	10	9.2550	3.4436
	20.0 mg	10	6.9500	3.9246
Post-treatment Degrees Flexion	0.5 mg	10	8.4750	5.4816
	5.0 mg	10	9.1500	4.2678
	10.0 mg	10	10.3800	4.3962
	20.0 mg	10	10.4250	9.1060
Pre-treament Clinical Score**	0.5 mg	10	9.5150	2.0146
	5.0 mg	10	8.7650	3.2873
	10.0 mg	10	9.7600	1.7807
	20.0 mg	10	9.9050	2.9799
Post-treatment Clinical Score	0.5 mg	10	9.2750	2.3674
	5.0 mg	10	10.6500	3.4311
	10.0 mg	10	10.8900	2.6811
	20.0 mg	10	11.5050	2.7454

*Relative viscosity was measured in a micro falling ball viscometer and reported in units of centistokes.

**Protein was measured with a Coomassie blue dye binding assay and reported in mg/ml.
[+]Means were calculated from averages of duplicate determinations of each point.

c. Statistical Analysis

The data from the study was analyzed by the linear plateau models described by Anderson and Nelson (Biometrics, 31: 303-318, 1975) to assess any dose response relationship between the measured parameters and the amount of Equron administered. The clinical observations analyzed consisted of the observations of heat, pain, swelling, and lameness. Each of these latter four factors ere ranked from 0 to 3 and the final clinical score was computed as the sum of the four of each animal at both pretreatment and post-treatment intervals. A decrease in the clinical score indicates a reduction in one or more of the parameters. A score of 0 represents the absence of any clinical sign of joint inflammation.

The outcome variable for the dose response analysis consisted of the observed differences between the post-treatment and pretreatment measures for both flexion and the clinical summary score. Additionally the two synovial fluid parameters, protein concentration and ln (relative viscosity) were analyzed as a function of dose for the difference between post-treatment and pretreatment values.

The data for all four groups was fit to three different models of dose response: linear model, plateau at 5 mg, and plateau at 10 mg. The model plateauing at the 10 mg dose level gave the best fit for clinical score and flexion. The change in relative viscosity, however, was best described by the linear model with the highest dose giving the greatest change. Synovial fluid protein showed no significant change across the dose groups for either of the models.

In figures 1 and 2 the means of the differences in clinical score and flexion are plotted as a function of dose. For both the clinical score and flexion, the basis for the plateau model selected is clearly seen. The means of the 10 and 20 mg groups are very similar.

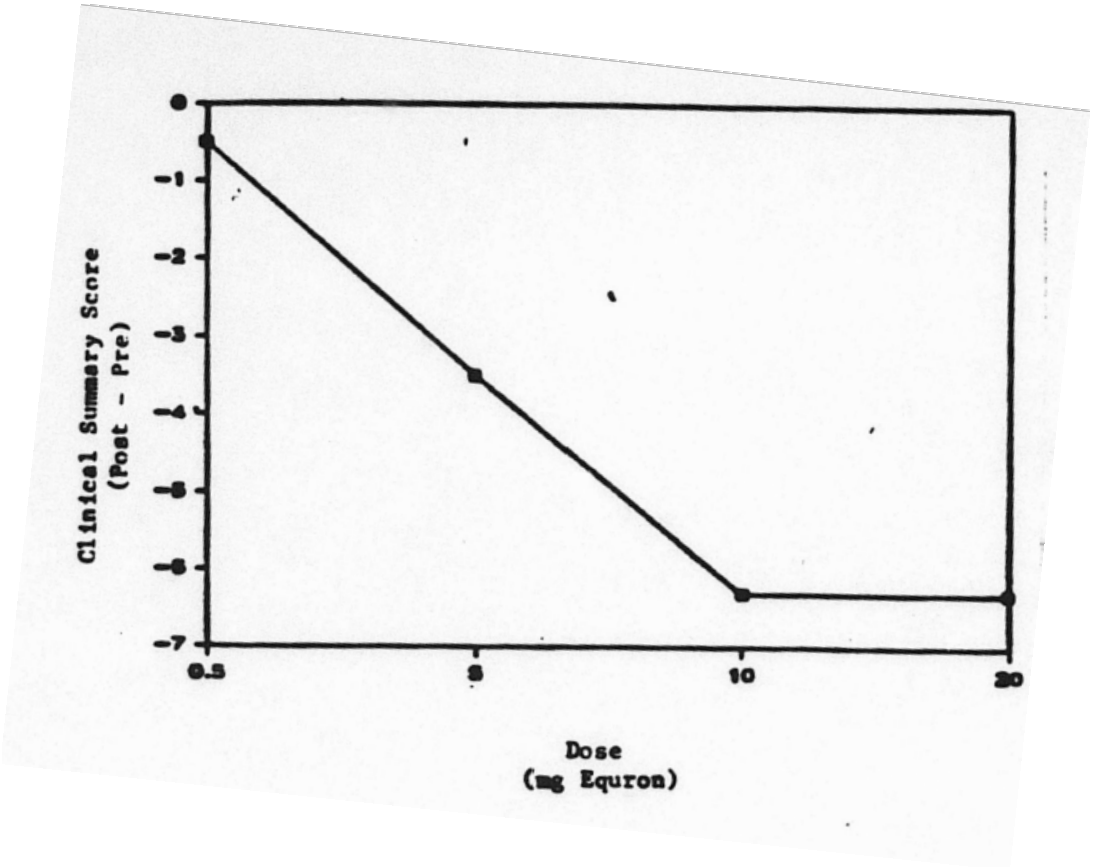

FIGURE 1: MEAN DIFFERENCE IN CLINICAL SCORE FOR THE 4 EQURON DOSE GROUPS

Plotted for each of the four dose groups (0.5 mg, 5 mg, 10 mg, and 20 mg) are the means of the differences between the post treatment and pretreatment clinical scores for each group. The clinical score for each case was calculated as the sum of a 0-3 ranking for heat, pain, swelling, and lameness. Maximum clinical score = 12 and minimum = 0. A difference of 0 between post and preclinical scores indicates no change and the larger the negative difference, the greater the clinical improvement.

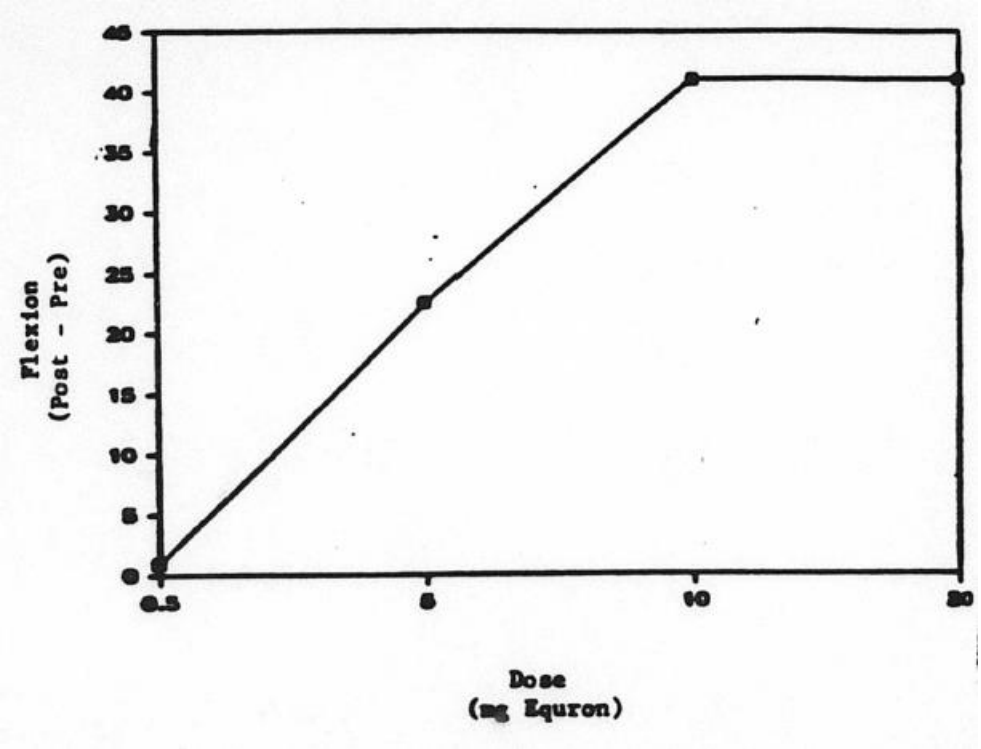

FIGURE 2: MEAN DIFFERENCE IN FLEXION FOR THE 4 EQURON DOSE GROUPS.

Plotted for each dose group are the mean difference (post-treatment) in degrees of flexion of the treated carpal joint. Flexion was measured in degrees relative to vertical. A difference of 0 indicates no change in pretreatment range of flexion.

d. Conclusion

Overall, these analyses demonstrate a positive dose response for 3 of the 4 measures in these animals. Clinical scores and flexion strongly support the 10 mg dose while relative viscosity indicates a linear relationship with increasing dosage. These data suggest that although the 20 mg dose produced the greater change in viscosity; the increase over that achieved by the 10 mg is not clinically relevant.

e. Adverse Reaction

No adverse reactions were noted in any of the clinical cases treated in this study.

2. Clearance Study

The clearance rate of various doses of Equron was determined in a study conducted between August 6 and August 24, 1984 at the Equine Gambit, Inc., Warwick, MD under the direction of Dr. Lawrence Cushing and associate Dr. Clark Cushing. Synovial fluid analyses were performed by Chesapeake Biological Laboratories, Inc., Hunt Valley, MD.

a. Study Design

The purpose of this study was to determine the clearance rate of Equron from the equine carpus and tibiotarsus as compared to an approved sodium hyaluronate product for equine joint use. Eight clinically normal thoroughbred horses between 4 and 8 years of age were used in the study providing 16 carpal and 16 tibiotarsal joints for evaluation. The 16 bilateral radiocarpal and tibiotarsal joints were randomly subdivided into 4 test groups per joint type as shown in the Table 2.

TABLE 2 DOSE GROUPS FOR CLEARANCE STUDY

Group	**#Joints/Group**	**Dose**
Carpus A	4	0.0 mg (saline)
Carpus B	4	10.0 mg Equron*
Carpus C	4	20.0 mg Equron
Carpus D	4	20.0 mg (approved product)**
Tibiotarsus AA	4	0.0 mg (saline)
Tibiotarsus BB	4	20.0 mg Equron
Tibiotarsus CC	4	40.0 mg Equron
Tibiotarsus DD	4	40.0 mg (approved product)

*Equron(TM) - 5 mg/ml sodium hyaluronate in saline
**Approved Product – 10 mg/ml sodium hyaluronate in phosphate buffered saline. At the start of the study (T=0 hours) a volume of synovial fluid equivalent to the volume of Equron to be injected was withdrawn from each test joint. These samples were analyzed for hyaluronate concentration to establish the pre-injection baseline values for this parameter. Immediately following the removal of this synovial fluid sample, the joint was injected with the assigned test material. Subsequently, synovial fluid samples were taken from each joint at 24, 48, 72, and 96 hours post-injection and analyzed for hyaluronate concentration.

b. Results

For both the carpal and tibiotarsal joints the injection of saline produced only slight changes in the endogenous hyaluronate levels at each of the sampling intervals (Tables 3 and 4). Elevations in synovial fluid hyaluronate concentration were observed 24 hours after injection for the other dosage groups with the increase being proportional to the amount of sodium hyaluronate injected. By 48 hours the hyaluronate concentrations were decreasing and by 72-96 hours they had dropped to essentially pre-injection levels for both the carpus and tibiotarsus.

TABLE 3 SYNOVIAL FLUID SODIUM HYALURONATE CONCENTRATION CARPAL JOINT

Dose	**0 Hours Post-Injection**	**24 Hours Post-Injection**	**48 Hours Post-Injection**	**72 Hours Post-Injection**	**96 Hours Post-Injection**
0 mg (saline)	1.088 ± .08 (4)	1.045 ± .08 (4)	1.068 ± .08 (4)	0.945 ± .08 (4)	1.036 ± .08 (4)
10 mg (Equron)	1.122 ± .03 (4)	1.356 ± .09 (4)	1.078 ± .01 (4)	1.105 ± .09 (4)	1.098 ± .13 (4)
20 mg (Equron)	.648 ± .09 (4)	.928 ± .13 (4)	.728 ± .08 (4)	.680 ± .06 (4)	.785 ± .03 (3)
20 mg (Approved Product)	1.231 ± .07 (4)	1.644 ± .10 (4)	1.271 ± .20 (4)	1.158 ± .08 (4)	1.189 ± .03 (4)

*Values shown are the mean ± SEM of the hyaluronate concentration in mg/ml for the number of joints shown in parenthesis. (A 5 mg dose was evaluated in the carpus but the injection was cleared so rapidly that accurate analysis was not possible).

TABLE 4 SYNOVIAL FLUID SODIUM HYALURONATE CONCENTRATION TIBIOTARSAL JOINT

Dose	**0 Hours Post-Injection**	**24 Hours Post-Injection**	**48 Hours Post-Injection**	**72 Hours Post-Injection**	**96 Hours Post-Injection**
0 mg (saline)	.673 ± .048 (2)	.696 ± .068 (3)	.708 ± .070 (4)	.620 ± .010 (4)	.74 ± .11 (3)
20 mg (Equron)	.743 ±.088 (3)	1.142 ±.184 (4)	.842 ±.050 (4)	.735 ±.068 (4)	1.098 ± .13 (4)
40 mg (Equron)	.674 ±.032 (4)	1.270 ±.039 (4)	1.053 ±.088 (4)	0.701 ±.042 (3)	.652 ±.056 (4)
40 mg (Approved Product)	.692 ±.065 (4)	1.445 ±.103 (4)	.908 ±.032 (4)	.758 ±.022 (4)	.617 ±.030 (4)

*Values shown are the mean ± SEM of the hyaluronate concentration in mg/ml for the number of joints shown in parenthesis.

c. Statistical Analysis

The first order rate constants for the clearance of sodium hyaluronate for each dosage group for the carpus and tibiotarsus were calculated from the slope of the line obtained from a plot of ln (hyaluronate concentration) versus time. Listed in Tables 5 and 6 are the individual rate constants for the intra-articular clearance of sodium hyaluronate from the synovial fluid of normal equine carpal and tibiotarsal joints. A one-way analysis of variance revealed no significant difference among the dosage rate constants for the carpal joints. Similarly no significance was found between the rate constants of the tibiotarsal joints.

TABLE 5 RATE CONSTANTS FOR INTRA-ARTICULAR CLEARANCE OF SODIUM HYALURONATE CARPAL JOINT

Dose Injected	Animal ID (joint)	Rate Constant (hours -1)	Mean ± S.D.
10 mg (Equron)	EG7 (RRC)	N/A**	0.0062 ± .0035
	EG7 (LRC)	0.0101	
	EG5 (RRC)	0.0034	
	EG5 (LRC)	0.0052	
20 mg (Equron)	EG9 (RRC)	.0039	.0063 ± .0026
	EG9 (LRC)	.0047	
	EG1 (RRC)	N/A**	
	EG1 (LRC)	.0101	
20 mg (Approved Product)	EG2 (RRC)	0.0055	0.0064 ± 0.0023
	EG2 (LRC)	0.0092	
	EG4 (RRC)	0.0072	
	EG4 (LRC)	0.0038	

*Rate constants were obtained from the slope of the line obtained from a plot of natural log concentration vs time (in hours) post-injection.
**N/A = not available. Insufficient sample at one time point precluded accurate rate analysis.

TABLE 6 RATE CONSTANTS FOR INTRA-ARTICULAR CLEARANCE OF SODIUM HYALURONATE TIBIOTARSAL JOINTS

Dose Injected	Animal ID (joint)	Rate Constant (hours -1)	Mean ± S.D.
20 mg (Equron)	EG3 (RRT)	0.0104	0.0079 ± .0043
	EG3 (LTT)	0.0126	
	EG5 (RRT)	0.0050	
	EG5 (LTT)	0.0036	
40 mg (Equron)	EG8 (RTT)	0.0082	0.0090 ± .0018
	EG8 (LTT)	0.0081	
	EG9 (RTT)	0.0081	
	EG9 (LTT)	0.0118	
40 mg (Approved Product)	EG10 (RTT)	0.0086	0.0114 ± .0021
	EG10 (LTT)	0.0135	
	EG11 (RTT)	0.0113	
	EG11 (LTT)	0.0120	

*Rate constants were obtained from the slope of the line obtained from a plot of natural log of concentration vs time (in hours) post-injection.

d. Conclusions:

These data indicate that there is not significant difference between the rates of clearance of 10 mg and 20 mg Equron from the equine carpus as compared to 20 mg of the approved sodium hyaluronate product. Similarly, the clearance rates of 20 mg and 40 mg of Equron were comparable to 40 mg of the approved product in the equine tibiotarsus. Although the hyaluronate concentration of the approved product was twice that of Equron, 10 mg/ml versus 5 mg/ml, there was not evidence for an effect of

product concentration on the rate constants which govern clearance. These data suggest that the appropriate frequency of repeated sodium hyaluronate injections into the diseased or injured joints is goverened by the kinetics of clearance.

3. Control Field Study

A controlled field trial of Equron was conducted at Freestate Racetrack, Laurel, MD between June 28 and September 9, 1985 under the direction of Dr. Paul Plante, Mitchellville, MD and associate Dr. Peter Alvarez. An approved sodium hyaluronate product was used as a positive control. Equron and control injections were performed by Dr. Plante and clinical evaluations were done by Dr. Alvarez. Sunovial fluid analyses were done by Chesapeake Biological Laboratories, Inc., Hunt Valley, MD.

a. Study Design

The purpose of the sudy was to compare the efficacy of Equron and an approved sodium hyaluronate product in treating equine joint dysfunction due to noninfectious synovitis associated with equine osteoarthritis. Clinical cases in training or racing demonstrating inflammation of the carpal or tibiotarsal joint were selected by the directing veterinarian. Selection criteria for the clinical cases entered into the study were: male or female, between 2 and 6 years of age, with inflammation of a carpus or tibiotarsus demonstrating lameness, heat, effusion, or pain upon flexion. Only one joint per animal was treated. Upon acceptance into the study each case was randomly assigned to one of 2 treatment groups: Equron or control. Dosages for the Equron group were: carpus 10 mg (5mg/ml x 2ml) and tibiotarsus 20 mg (5mg/ml x 4 ml). For the control group the dosages were: carpus 20 mg (10mg/ml x 2ml) and tibiotarsus 40 mg (10mg/ml x 4ml). Prior to treatment each case was graded for lameness, heat, pain and swelling and a synovial fluid sample obtained for determination of relative viscosity and protein concentration. A single intra-articular injection of either Equron or control was administered and at 6-8 days post-injection clinical evaluations and synovial fluid samplings were repeated. If the animal was not sound a second injection of the original product (Equron or control) could be given. Up to 4 weekly injections of either product could be given. Both the clinical evaluations of the cases treated and the synovial fluid analyses were conducted blind.

b. Results:

The 40 carpal cases evaluated in the study were characterized prior to treatment and varying degrees of lameness, swelling, heat and pain in the affected carpal joint. Although not all of the clinical signs of inflammation were present in all cases, each animal was judged by the evaluating practitioner as exhibiting clinical inflammation of the carpus.

The study protocol allowed for a maximum of 4 weekly injections of either product at the discretion of the evaluating veterinarian. In the Equon group of 20 clinical cases, 14 received one injection, 2 received 2 injections, 2 received 3 injections, and 2 received 4 injections. By the end of treatment regimen 14 of the 20 cases were judged to have shown

maximum improvement (76-100%). In each case the lameness score was graded 0 at the end of the treatment. The other parameters, swelling, heat, and pain showed a general decrease within the group, although in some cases not all three changed. Six of the 20 cases in the Equron group were judged to have improved 50-75% at the end of their treatment regimen. Similar to those cases with maximal improvement, these cases showed an overall clinical reduction in lameness and inflammation. None of the cases in this group were considered treatment failures. In all cases Equron treatment resulted in substantial clinical improvement and the injections were well tolerated with no post-injection reactions observed.

The changes observed in synovial fluid relative viscosity and protein reflected the general clinical efficacy of Equron. Eighteen of the 20 cases showed an increases in relative viscosity at the end of treatment. In one case the pretreament relative viscosity was very high and at the end of treatment it had decreased but was still well above normal. The post treatment changes in protein value were somewhat more varable since some of the cases were normal in this respect prior to treatment. Of ten cases with elevated protein (>10mg/ml) prior to Equon injection seven showed a decrease in this parameter at the end of treatment.

Comparison of the effect of the control injection in the second group of 20 clinical cases showed a marked similarity in clinical efficacy to that achieved with Equron. Seventeen cases received one injection, 2 received 2 injections and one reveived 4 injections. At the end of the treatment eleven of the cases were judged maximally improved (76-100%) and eight were judged 50-75% improved. Only one case was considered a poor response and that was due to a slab fracture which occurred during but unrelated to control treatment. Similar to the Equron group, reductions in lameness, swelling, heat and pain, paralleled the overall clinical improvement.

Analysis of the synovial fluid of this group revealed a general increase in relative viscosity throughout and a variable decrease in protein concentration. Post treatment relative viscosity increased in seventeen of the 20 cases. In three cases it decreased. Of the ten cases with elevated synovial protein (>10mg/ml) prior to treatment, five showed a decrease after treatment.

Comparison of the relative efficacy of Equron and control in the tibiotarsal joint was conducted identically to the carpal evaluations but with 10 cases per group. Because of the anatomical differences between the tarsus and carpus, the principal clinical sign of inflammation was swelling with little lameness, heat or pain. These are typical of tibiotarsal problems in racing horses.

Comparing the clinical data for the Equron and control tibiotarsal groups shows a striking similarity in resolution of swelling and lameness when present. In the Equron group eight of the 10 cases were judged 76-100% improved after a single injection, and all 10 of the control treated cases were maximally improved at the same period.

The synovial fluid data for the Equron group revealed that relative viscosity in seven of the 10 cases was increased after one injection. Only two of the cases had elevated protein (>10mg/ml) prior to treatment and both had decreased. In the conrol group nine of the ten cases showed increases in relative viscosity and all of the ten cases showed increases in relative viscosity and all of the five cases with elevated protein showed a decrease at one week post-treatment.

Similar to the results fo the carpal data, there is no apparent difference between the two products in resolving the typical tibiotarsal problems in these animals. All injections were well tolerated with no adverse reactions.

A summary of the clinical and synovial fluid changes observed in this study is given in Tables 7A and 7B. These tables give the means and one standard deviation for the summary clinical score, synovial fluid relative viscosity,and protein content before and at the end of the treatment for the carpal and tibiotarsal joints. As is evident form the data there is little difference between products in the changes observed in any of the parameters. For the carpal joints (Table 7A), the summary clinical score for both groups (Equron and control) were essentially equivalent at the end of the treatment. Similarly the increase in synovial fluid viscosity achieved by either product were the same. The large standard deviation seen in this parameter reflect a few cases in both groups which achieved extremely high post treatment values. In both the Equron and control group little change in synovial protein concentration was observed since the clinical cases were largely normal in this respect prior to treatment.

Changes in the summary clincal score and synovial fluid relative viscosity and protein concentration over the treatment regimen for tibiotarsal joints (Table 7B) are similar to that observed with the carpal joints. In both groups the clinical scores decreases markedly by the end of treatment. Relative viscosity increases in both groups were equivalent with considerable overlap between the two. Synovial fluid protein remained essentially unchanged.

From a qualitative standpoint, there appears to be no marked difference in the clinical efficacy of the two products in resolving inflammation of the carpal or tibiotarsal joints. Because to the relatively small numbers in both groups, a difference of one or two cases achieving maximum improvement, no lameness, etc. is probably not significant.

The difference in the number of repeated treatments given (Equron 6 and control 3) most likely reflects differences in the extent of chronic involvement in some of the clinical cases. This consideration however cannot be quantitated adequately in this study to justify detailed comparison of the frequency of repeat injections.

TABLE 7A MEAN AND STANDARD DEVIATIONS FOR EFFICACY PARAMETERS CARPAL JOINTS

EQURON GROUP n = 20	**PRETREATMENT**	**END OF TREATMENT**
Summary Clinical Score*	4.35 ± 1.39	0.95 ± 0.89
Relative Viscosity**	29.27 ± 62.87	101.60 ± 172.85
Protein***	12.20 ± 4.47	11.90 ± 4.43
% Improvement[+]		14 (76-100%)
# cases/ranking		6 (50-75%)
		0 (0-49%)

CONTROL GROUP n = 20	**PRETREATMENT**	**END OF TREATMENT**
Summary Clinical Score*	4.30 ± 2.18	0.79 ± 1.03[++] (n = 19)
Relative Viscosity**	24.01 ± 41.98	100.87 ± 83.00
Protein***	10.85 ± 4.17	12.24 ± 4.67
% Improvement[+]		11 (76-100%)
# cases/ranking		8 (50-75%)
		0 (0-49%)

*Clinical score is the sum of ranking (0 3) for heat, pain, swelling and lameness. (0 = no evidence; 1 = slight; 2 = moderate and 3 = severe). Maximum clincal score = 12 and minimum clincal score = 0.
**Relative viscosity was measured in a micro falling ball viscometer and reported in units of centistokes.
***Protein was measured with a Coomassie blue dye binding assay and reported in mg/ml.
[+]% Improvement was ranked as 0-49%, 50-75% and 76-100% at the end of treatment.
[++]Case #41 developed a slab fracture of the treated joint during treatment and was not used in these calculations.

TABLE 7B MEAN AND STANDARD DEVIATIONS FOR EFFICACY PARAMETERS TIBIOTARSAL JOINTS

EQURON GROUP n = 10	**PRETREATMENT**	**END OF TREATMENT**
Summary Clinical Score*	2.8 ± 1.03	0.4 ± 0.52
Relative Viscosity**	7.53 ± 5.46	9.98 ± 6.37
Protein***	9.47 ± 2.78	10.26 ± 4.19
% Improvement[+]		10 (76-100%)
# cases/ranking		0 (50-75%)
		0 (0-49%)

CONTROL GROUP n = 20	**PRETREATMENT**	**END OF TREATMENT**
Summary Clinical Score*	2.4 ± 0.84	0.1 ± 0.32
Relative Viscosity**	5.61 ± 3.34	9.68 ± 5.17^{++}
Protein***	10.08 ± 2.68	7.41 ± 1.90
% Improvement^{+}		10 (76-100%)
# cases/ranking		0 (50-75%)
		0 (0-49%)

*Clinical score is the sum of ranking (0 3) for heat, pain, swelling and lameness. (0 = no evidence; 1 = slight; 2 = moderate and 3 = severe). Maximum clincal score = 12 and minimum clincal score = 0.
**Relative viscosity was measured in a micro falling ball viscometer and reported in units of centistokes.
***Protein was measured with a Coomassie blue dye binding assay and reported in mg/ml.
$^{+}$% Improvement was ranked as 0-49%, 50-75% and 76-100% at the end of treatment.
$^{++}$This value did not include case #39 which had an end of treatment relative viscosity approximately 10 fold greater than the mean of the other 9 cases in this group. Inclusion of case #39 would give 18.09 ± 27.03.

c. Statistical Analysis

The data from this study was statistically analyzed by Dr. Terri Beaty, The Johns Hopkins University, by a two tailed T test at the .05 level.

The hypothesis tested is the null hypothesis: the control product is different from Equron in its efficacy. The two tailed T test employed would detect a significant difference of measured efficacy between the two products if the control is more or less effective than Equron.

At pre- and post-treatment, swelling, heat, pain or pressure and lameness were graded on an ordinal scale of 0 to 3. Synovial fluid relative viscosity and protein concentration were determined in centistokes and mg/ml respectively.

For the purpose of comparing the efficacy of the contol product relative to Equron the four clinical measures (swelling, heat, pain and lameness) were summed to give a single summary clinical score. The outcome variables for statistical analysis were the difference in 1 week post and pre-treatment for summary clinical score and the log (ratio) of 1 week and pre-treatment for relative viscostity and protein concentration.

Since only a few clinical cases in each group (control and Equron) received more than one injection; statistical ananlysis taking into account the frequency of repeated injections was not justified. For this reason statistical comparison of the two products was limited to the outcome variables determined before and after 1 week of treatment.

The results of the statistical analysis are shown in Table 8A for the 40 animals with tested carpal joints and in Table 8B for the 20 animals with

tested tibiotarsal joints. There was no signififcant difference between the two treatment groups (the Equron and the control groups) using the response to treatment for these three measured in either joint.

TABLE 8A CARPAL JOINT

	Equron (n=20)	**Control (n=20)**	**t-test (38 dif)**	**2-tal p**
Difference in Clinical score (1 wk-pre value)	-2.45*	-3.15	1.37	.18
	1.23**	1.93		
	0.28***	0.43		
ln (viscosity 1 wk -) –ln (viscosity pre-)	1.29	1.08	0.52	.61
	1.21	1.23		
	0.27	0.28		
ln (protein 1 wk-) –ln (protein pre-)	-0.05	0.12	-1.61	.12
	0.23	0.37		
	0.05	0.08		

Response to treatment in 40 animals with treated carpal joints. The mean values (*), standard deviations (**), and standard errors of mean (***) for two groups of 20 animals each with tested carpal joints are given. T statistics and p values for a two-tailed test are also given.

TABLE 8B TIBIOTARSAL JOINT

	Equron (n=10)	**Control (n=10)**	**t-test (18 dif)**	**2-tal p**
Difference in Clinical score (1 wk-pre value)	-2.40*	-2.30	-.25	.81
	0.84**	0.95		
	0.27***	0.30		
ln (viscosity 1 wk -) –ln (viscosity pre-)	0.33	0.82	-1.36	.19
	0.69	0.90		
	0.22	0.28		
ln (protein 1 wk-) –ln (protein pre-)	0.05	-0.31	1.78	.09
	0.48	0.44		
	0.15	0.14		

Response to treatment in 20 animals with treated tibiotarsal joints. The mean values (*), standard deviations (**), and standard errors of mean (***)for two groups of 10 animals each tested tibiotarsal joints are given. T statistics and p values for a two tailed test are also given.

B. Corroborative Studies

Three uncontrolled field trials of Equron were conducted in support of the pivotal studies: two in the US and one in England and Ireland. The names and addresses of the participating investigators in these trials are given in the appendix.

1. Preliminary Uncontrolled Field Trial

   A preliminary clinical evaluation of Equron was conducted between August 15 and October 30, 1982 to evaluate various methods of data collection and an assessment of investigators who could participate in the large scale clinical study. The study was conducted with material produced in the laboratory for testing purposes. The clinical cases were chosen from horses having degenerative or traumatic inflammation joint problems at the time of entry into the study and were restricted to carpal, fetlock and tibiotarsal. Only one joint per case was treated. Horses with known or suspected infections, known or suspected acute fractures or chips, or any steriod injections in the joint to be treated within four weeks of entry into the study were not included.

   Efficacy of the drug was evaluated throughtout the clinical evaluations by the attending practitioner. No synovial fluid analysis was performed. The product was administered intra-articularly as a single 10 mg injection. The clinical condition of the joint was evaluated at the time of Equron injection, 24 hours post-injection and subsequently at 7-10 days and 14-17 days post injection. In addition to evaluations of any decrease in lameness after treatment, the practitioners were requested to report any side effects (post injection reactions).

   Of approximately 200 cases selected, 135 completed the evaluation program. Of the conditions treated the majority were chronic with an average pre-treatment lameness score of 2 out of a possible 4 degrees. The overall response to treatemnt was judged as excellent in 77 of the 135 clinical cases. Another 33 were graded as good and 12 as fair. Poor response was indicated in only 2 cases. Six reported no change in pre-treatment condition.

   No significant adverse reactions were reproted. In 16 cases slight post-injection swelling was recorded by the attending veterinarian. Ten of these were from the same practitioner who reported some slight edema and pain after injection yet considered the overall response to the drug in these cases as excellent. None of the reported side effects were considered serious and all resolved within 24 to 48 hrs.

2. Uncontrolled Field Trial

   A large scale uncontrolled field trial was conducted in the summer and fall of 1984 in the US involving 18 equine practices and 159 clinical cases. The results of this study have the most relevance to the clinical application of Equron in that the investigators were required to evaluate, in extensive detail, each case that was treated. Names and addresses of the participating veterinarians are listed in the appendix. Equron was adminsitered intra-articularly into the affected joint in a treatment regimen of single injections at two week intervals. Up to four injections (10 mg/2 ml - carpus and fetlock and 20 mg/4ml - tibiotarsus) were given at the discretion of the attending veterinarian. Clinical improvement was assessed by clincal evaluations made at weekly intervals and changes in synovial fluid relative viscosity and protein concentration were determined by a bi-weekly laboratory analyses of synovial fluid samples. Synovial fluid testing was performed by Chesapeake Biological Laboratories, Hunt Valley, MD.

The results of this study are summarized in the following tables. The percent improvement achieved at the end of treatment for unilateral and multilateral treated cases is given in Table 9. For unilaterally treated cases 63% displayed improvement of 76-100% while 79% fo the multilaterally treated cases showed a similar improvement. Table 10 shows the same data arranged by joint type treated. By the end of the study 83% and 84% respectively for carpal and tibiotarsal joints had achieved 76-100% improvement. Less impressive but still highly positive results were observed with fetlock joints of which 51% showed similar improvement.

TABLE 9 PERCENT IMPROVEMENT AT END OF STUDY FOR UNILATERALLY AND MULTILATERALLY TREATED HORSES

INDEX RANGE	UNILATERAL*	MULTILATERAL**	COMBINATION
76-100%	42	65	107
51-75%	12	7	19
26-50%	8	3	11
0-25%	5	7	12
NOT RECORDED***	5	5	10

*Unilateral - one joint per case treated
**Multilateral - multiple joints per case treated
***In a few of the submitted cases, the veterinarian failed to record his observation for one of the parameters. In the following table these are indicated as not recorded.

TABLE 10 PERCENT IMPROVEMENT AT END OF THE STUDY FOR CARPAL, FETLOCK AND TIBIOTARSAL JOINTS

INDEX RANGE	CARPAL	FETLOCK	TIBIOTARSAL
76-100%	54	27	26
51-75%	3	12	4
26-50%	6	4	1
0-25%	2	10	0
NOT RECORDED***	5	3	2

*See footnote Table 9

Similar to the percent improvements observed , there was a marked reduction in lameness as a result of Equron treatment. Table 11 shows the distribution of lameness grading for unilaterally and multilaterally treated joints before the start and at the end of the study. As illustrated there were substantial reductions in lameness over the course of the study with 71% of the combined cases exhibiting no lameness at the end of the study. Table 12 shows the same results according to joint type. The data suggest there is no relevant difference in efficacy for the 3 joints.

TABLE 11 DISTRIBUTION OF LAMENESS VALUES AT THE START AND THE END FOR UNILATERALLY AND MULTILATERALLY TREATED CASES NUMBER OF JOINTS WITHIN INDEX RANGE FOR TREATMENT PERIOD

Index Value	Before Start of Study – Uni*	Before Start of Study – Multi**	Before Start of Study – Comb	At End of Study – Uni	At End of Study – Multi	At End of Study – Comb
3	11	3	14	2	0	2
2	29	31	60	5	2	7
1	29	43	72	19	16	35
0	1	10	11	44	69	113
Not Recorded+	2	0	2	2	0	2

*Unilateral - one joint per case tested
**Multilateral - multiple joints per case treated
***Lameness was graded on a scale of 0 3: 0 = none, 1 = slight head nod at walk; 2 = beginning head nod, decreased anterior phase; 3 = noticeable head nod, decreased weight bearing at rest.
+See footnote Table 9

TABLE 12 DISTRIBUTION FO LAMENESS VALUES AT THE START AND THE END OF THE STUDY FOR CARPAL, FETLOCK, AND TIBIOTARSAL JOINTS NUMBER OF JOINTS WITHIN INDEX RANGE FOR TREATMENT PERIOD

Index Value	Before Start of Study – Carpal	Before Start of Study – Fetlock	Before Start of Study – Tibio-Tarsal	At End of Study – Carpal	At End of Study – Fetlock	At End of Study – Tibio-Tarsal
3	3	8	3	1	1	0
2	27	22	11	3	3	1
1	39	19	14	15	17	3
0	0	6	5	50	34	29
Not Recorded+	1	1	0	1	1	0

***Lameness was graded on a scale of 0-3: 0 = none, 1 = slight head bob at walk; 2 = beginning head nod, decreased anterior phase; 3 = noticeable head nod, decreased weight bearing at rest.
+See footnote Table 9.

Measurements of changes in synovial fluid protein concentration and relative viscosity substantiate the observed clinical efficacy. Nineteen of the treated joints (10 carpals, 5 fetlock, and 4 tibiotarsal) exibited elevated synovial protein indicative of acute inflammation prior to treatment. For these cases the differences in synovial protein concentration at the start and at the end of the study were determined and analyzed by the Wilcoxon rank test. A statistically significant decrease in protein concentration ($p<0.005$) was associated with Equron treatment. Reduced relative viscosities of the synovial fluid was observed in 51 cases at the beginning of the study (18 carpal and 33 fetlock).

By the end of the study a Wilcoxon rank test revealed a significant increase ($p<0.025$) in relative viscosity for these cases.

At the completion of the study the participating veterinarians graded the overall clinical response of Equron treatment as excellent, good, fair, poor, or none for each clinical case. Tables 13 and 14 give the percentage for each grade for unilaterally and multilaterally treated cases and for joint types. Table 15 gives the overall results of Equron treatment with regard to the number of treatments per joint. Of the 55 joints treated only once with Equron 52 joints (95%) received a final evaluation of an excellent or good response. For those 51 joints receiving two injections of Equron a total of 43 (84%) were given an excellent or good evaluation. The corresponding numbers for joints treated 3 and 4 times with Equron were 21 of 22 (95%) and 28 of 31 (90%), respectively, which were rated excellent or good responses. Overall 144 cases out of 159 were judged as excellent to good response giving a total improvement of 91%.

These findings indicate that, when the number of Equron treatments is at the discretion of the veterinarian, a high degree of clinical efficacy for the drug pertains, irrespective of the final number of injections.

TABLE 13 FINAL EVALUATIONS FOR UNILATERAL OR MULTILATERAL TREATED CASES

Final Evaluation Joint	Final Evaluation Excellent No.	Final Evaluation Excellent %	Final Evaluation Good No.	Final Evaluation Good %	Final Evaluation Fair No.	Final Evaluation Fair %	Final Evaluation Poor No.	Final Evaluation Poor %	Final Evaluation None No.	Final Evaluation None %
Total (n=159)	113	71%	31	19%	6	3.8%	1	0.6%	8	5.0%
Uni (n=72)	46	64%	18	25%	3	4.2%	1	1.4%	4	5.6%
Multi (n=87)	67	77%	13	15%	3	3.4%	0	0%	4	4.6%

*The criteria applied to making these evaluations were as follows:

- Excellent: Returned to racetrack or full riding work. Capable of work without recurrence for more than two weeks.
- Good: Returned to training or riding: duration of effect is one to two weeks.
- Fair: Improvement, but not significant for regular training or riding.
- Poor: Improvement is less than one week duration.
- None: No change

TABLE 14 FINAL EVALUATION FOR CARPAL, FETLOCK, AND TIBIOTARSAL JOINTS

Final Evaluation Joint	Final Evaluation Excellent No.	Final Evaluation Excellent %	Final Evaluation Good No.	Final Evaluation Good %	Final Evaluation Fair No.	Final Evaluation Fair %	Final Evaluation Poor No.	Final Evaluation Poor %	Final Evaluation None No.	Final Evaluation None %
Carpal (n=70)	53	76%	13	19%	3	4.3%	1	1.4%	0	0%
Fetlock (n=56)	31	55%	14	25%	3	5.4%	0	0%	8	14%
Tibio-Tarsal (n=33)	29	88%	4	12%	0	0%	0	0%	0	0%

TABLE 15 FINAL EVALUATIONS OF EQURON BASED ON THE NUMBER OF INJECTIONS PER TREATED JOINT FINAL EVALUATION

No. of Infections	Final Evaluation Excellent No.	Final Evaluation Excellent %	Final Evaluation Good No.	Final Evaluation Good %	Final Evaluation Fair No.	Final Evaluation Fair %	Final Evaluation Poor No.	Final Evaluation Poor %	Final Evaluation None No.	Final Evaluation None %
ONE [n=55]	46	84%	6	11%	2	3.6%	1	1.8%	0	0%
TWO [n=51]	33	65%	10	20%	1	2.0%	0	0%	7	14%
THREE [n=22]	17	77%	4	18%	1	4.5%	0	0%	0	0%
FOUR [n=31]	17	55%	11	35%	2	6.5%	0	0%	1	3.2%

Overall the response to Equron treatment was considered excellent. Mild post-injection swelling was noted in 7 (4%) of the 159 total joints treated. In all cases these reactions were transient and resolved within 48 hours.

3. United Kingdom and Ireland Trials

   A study was conducted by the International Animal Health Division of E.R. Squibb & Sons, Inc. to confirm clinical efficacy for registration of Equron in the U.K. and Ireland, during the spring of 1984. Equron was administered to 24 clinical cases diagnosed as having equine joint disease by veterinarians in six practices in the U.K. and Ireland during spring 1984. The product was administered as a single, 10 mg (2ml) dose injected into the stifle, carpal, fetlock, or tibiotarsal joint.

   Each horse was assessed before treatment and twice after treatment using the lameness score system from the U.S. trial.

Lameness Score	Percent of Horses Pretreatment	Percent of Horses 7 Days Posttreatment	Percent of Horses 14 Days Posttreatment
3	11	0	0
2	37	19	18
1	48	44	12
0	4	38	70

Final assessments of therapeutic response were given in 23 of 24 clinical cases treated. Of these 8 (35%) were judged as excellent; 6 (26%) as good; 5 (22%) as fair; 2 (9%) as poor; and 2 (9%) as no response. Overall 19 cases (83%) demonstrated positive clinical response to Equron treatment. Based on these and other relevant studies conducted in the U.S., Equron was granted approval in both the U.K. and Ireland on October 10, 1984. (Registration approval 0034/4035).

III. TARGET ANIMAL SAFETY

A. Pivotal Studies

1. Acute Toxicity Study

An acute toxicity study was conducted between September 1 and November 14, 1983 at the Rochester Equine Clinic, Rochester, NH under the direction of Dr. Grant Myhre, clinic director. Hematologies, blood and serum chemistries, and urinalysis were done in duplicate by the Rochester Equine Clinic and Metpath Laboratories, Teterboro, NJ. Synovial fluid analyses were done by Chesapeake Biological Laboratories, Inc., Hunt Valley, MD.

a. Study Design

The purpose of this study was to evaluate the safety of acute administration of Equron injected intra-articularly into the equine joint. Six mixed bred horses (5 geldings and 1 mare) between 5 and 11 years of age received intra-articular injections of 4 sequential 100 mg/doses per animal. Equron injections were given at 2 week intervals for 8 weeks. Each animal received 20 mg and 30 mg in both carpal and tibiotarsal joints respectively at each injection period. Each animal was acclimated for 14 days during which physical examinations and blood urine and synovial samples wer collected to establish base line values. Upon commencing the Equron injections, physical examinations, hematology, serum chemistries and urinalysis were performed on days 0, 2, 4, 7, 14, 28, 42, and 56. Synovial fluid samples were obtained prior to each injection at days 0, 14, 28, 42 and 2 weeks after the last injection on day 56.

The physical examinations consisted of clinical observations to detect adverse reactions affecting the ocular, equilibrium, musculature, appetite, integument, gastrointestinal, cardiovascular, respiration and general behavior. The injection site was monitored for 24 hours after each injection for signs of local inflammation. Clinical chemistries encompassed hematology - WBC, RBC, differential WBC, PCV, MCV, MCHC, hemaglobin, prothrombin time, activated clotting time, sedimentation rate, and platelet count; blood and serum chemistries - CO2, SDH, BSP, creatinine, glucose, Ca, P, LDH, SGOT, total protein, albumin, globulin, and total bilibrubin; urinalysis - color, consistency, quantity, specific gravity, pH, protein, glucose, bilibrubin, urobilinogen, WBC, RBC, epithelial cells and formed elements. Synovial fluid analysis consisted of measurements of relative viscosity, protein and hyaluronate concentration, "mucin clot" quality and WBC content.

b. Results

Over the course of the 8 week acute toxicity study no general adverse effects of Equron administration were observed. Examination of the data from this study gave no indication of adverse effects either systemically or locally arising from repeated intra-articular injections of Equron. The physical examinations throughout the testing periods were unremarkable. No significant physical changes were observed in any of the animals which would indicate systemic toxicity. All animals remained in good health throughout the study.

The findings of the physical examinations were in agreement with the results of hematology, blood and serum chemistry, and urinalysis testing. No significant changes were observed in any of the measured parameters which would indicate systemic toxicity. Evaluations made during the pretreatment acclimation period exhibited random variations which were present throughout the study. In general the variations between animals were consisently greater than that observed for any animal throughout the study. When an animal was outside the normal range in a specific test at one testing period it would generally be within the normal range at the next testing interval. No trends in any of these laboratory analyses were observed which would indicate systemic disorders. At the completion of the study all animals were judged normal. Post-injection joint effusions were noted in several cases but appeared random and not consistent within either a particular animal or series of injections. Considering the large number of intra-articular samplins and/or injections these incidences of post-injectionost-injection effusions were not considered significant. Of a total of 168 arthrocenteses and 256 post-injection examinations, 32 post-injection swellings were observed. In all cases they were quite transient and resolved within 24 to 48 hours. There was no evidence of lameness and the range of motion remained normal. Due to the random occurrence of these post injection effusion, they were considered to result form the arthrocentesis and not directly related to the injection of sodium hyaluronate.

The results of the repeated synovial fluid analyses were consistent with those from the physical examination and clinical chemistries in that there was no evidence for intra-articular inflammation arising from the repeated Equron injections. Some minor changes in synovial fluid properties relative to pretreatment values were observed but these were not consistent with synovial inflammation. Figures 3 and 4 show the results of analysis of synovial fluid protein and 1n relative viscosity form the tibiotarsus of the experimental animals. For both parameters the sequential evaluations reveal a minor change relative to pretreatment values. Synovial protein concentration showed a slight rise following the first Equron injection and remained slightly elevated throughout the study period. Relative viscosity showed a minor decrease after the first injection and thereafter remained essentially unchanged for the remainder of the study. These small changes were not indicative of significant synovial inflammation and probaby arose from the trauma of repeated arthrocentesis. Similar small changes were observed in the synovial fluid from the carpal joint.

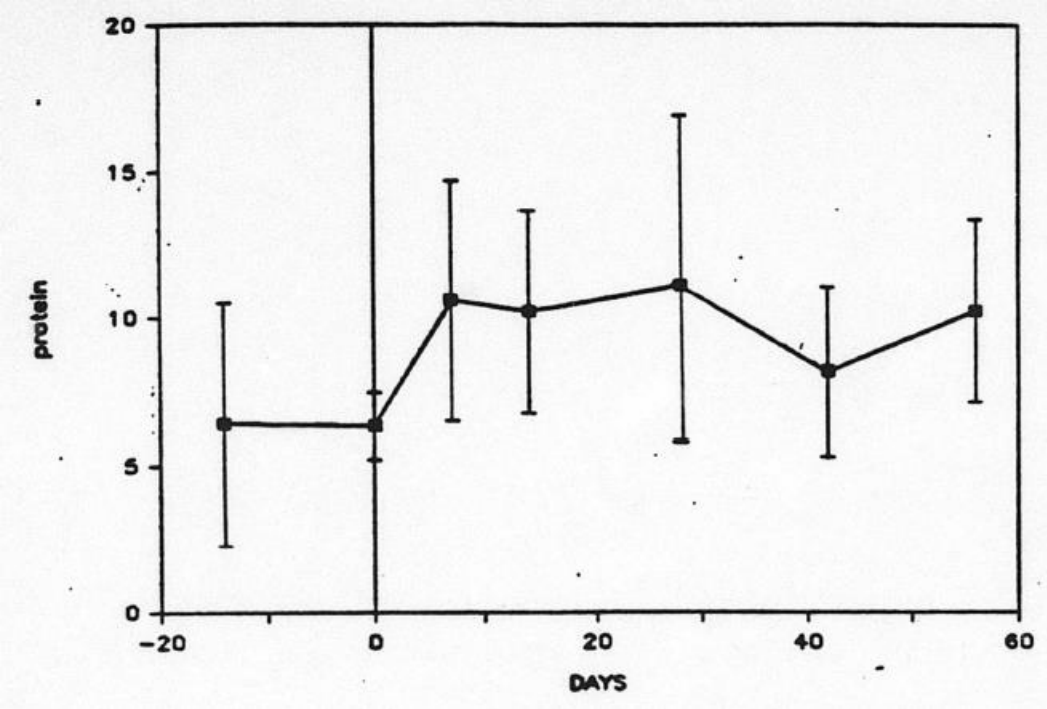

FIGURE 3: ACUTE TOXICITY: MEAN + STANDARD DEVIATION FOR TIBIOTARSAL SYNOVIAL FLUID PROTEIN CONCENTRATION.

Plotted are the means ± one standard deviation for tibiotarsus synovial fluid protein concentration in mg/ml on the days indicated during the acute toxicity study of Equron. The data shown for days -14, and 0 represent pretreatment values. Equron injections (30 mg/tibiotarsus) were given immediately following synovial fluid sampling on days 0, 14, 28 and 42. The means are comprised of 12 values (right and left tibiotarsus of 6 horses).

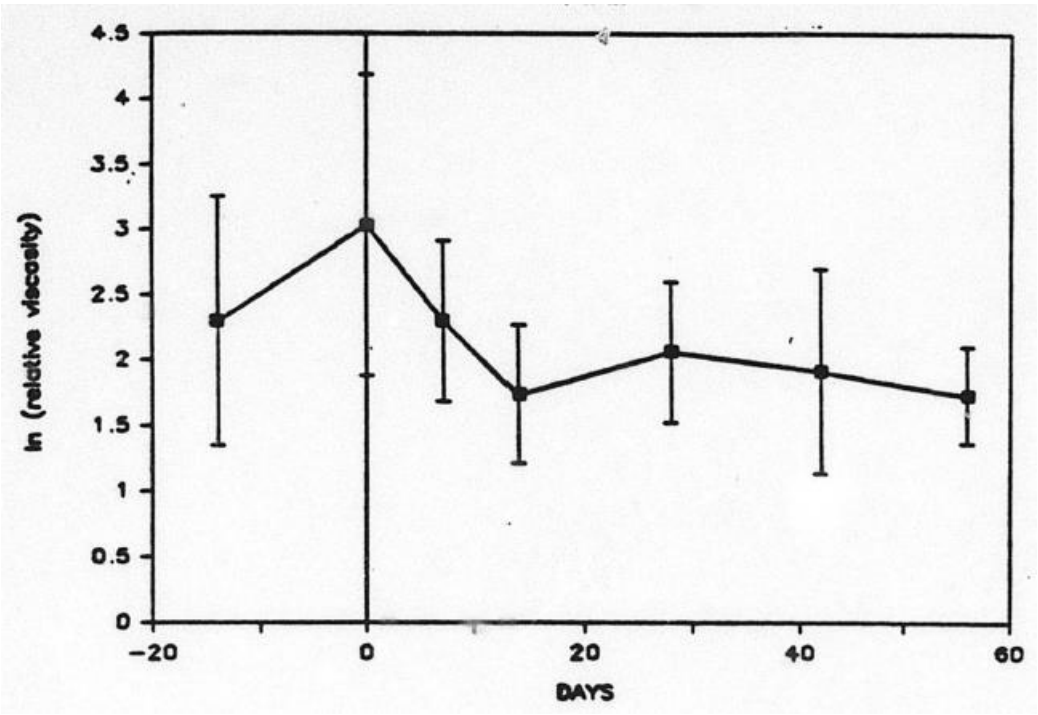

FIGURE 4: ACUTE TOXICITY: MEANS ± STANDARD DEVIATION FOR TIBIOTARSAL 1N (RELATIVE VISCOSITY).

The relative viscosity of the synovial fluid form the right and left tibiotarsus of each of six animals was measured in centistokes and converted to natural log for plotting. The data is presented as described for protein concentration in Figure 4. 1n = natural log.

c. Statistical Analysis

Statistical evaluation of the data was performed by Dr. Terri H. Beaty of the Johns Hopkins University School of Medicine, Baltimore, MD. Seven clinical parametersss, WBC, MCV, sedimentation rate, SDH, glucose, total protein, and globulin were analyzed for possible trends either over time or among individual animals by examining box plots of the variables and using a robust two way analysis of variance. In general the variables among animals were greater than any variation over time for each parameter. No significant trends were observed which would suggest any toxic effets of acute Equron administration.

Although the synovial fluid values did show some minor changes over the couse of the study, they were not considered clinically relevant and thus no statistical evaluation was warranted.

d. Conclusions

The data from this study demonstrate that repeated intra-articularly administrered Equron is free from systemic toxicity.

e. Adverse Reactions

No adverse reactions were observed in any of the experimental animals thoughout the study.

2. Chronic Toxicity Study

A chronic toxicity study was conducted at the Rochester Equine Clinic, Rochester, NH under the direction of Dr. Grant Myhre, clinic director between November 28, 1983 and June 4, 1984. Hematologies, blood and serum chemistries, and urinalyses were done in duplicate by the Rochester Equine Clinic and Metpath Laboratories, Teterboro, NH. Synovial fluid analysis was performed by Chesapeake Biological Laboratories, Hunt Valley , MD. Histopathology was done by Dr. Roger Wells, board certified pathologist, Veterinary Diagnostic Laboratory, University of New Hampshire, Durham, NH.

a. Study design

The purpose of this study was to evaluate the safety of chronic administration of Equron injected intra-articularly into the equine joint. Six mixed bred horses (3 geldings and 3 mares) between 6 and 10 years of age received 12 sequential intra-articular injections at 2 week interval for 24 weeks. Each experimental animal received 40 mg/4 ml Equron in the right radiocarpus and 80 mg/10 ml Equron in the right tibiotarsus. The left radiocarpus and tibiotarsus received equivalent volumes of sterile, pyrogen free saline as controls. Physical examinations were conducted at days 0, 1, 2, 4, 7 and 14 and thereafter at 14 day intervals throughout the 168 day study. Blood and urine samples were obtained for analysis at the same intervals. Synovial fluid samples were obtained at days 0, 7 and 14 and thereafter at 14 day intervals throughout the study.

At termination of the study, three of the six horses were euthanized for gross and histopathological examination. The entire animal was examined grossly and specific tissues collected and examined microscopically. The injected joints, both Equron and control, were examined grossly and representative tissue samples obtained for microscopic evaluation. This latter histopathology was conducted blind.

Physical examinations, (hematology, blood and serum chemistries, urinalysis and synovial fluid analyses) were identical to those performed in the acute toxicity study. Serum alkaline posphatase was included in the serum chemistries as a measure of liver function.

b. Results

The results of the chronic toxicity evaluation are consistent with those of the acute toxicity study. The results fo hematology, blood and serum chemistries and urinalyses gave no evidence of any toxic effect arising from the chronic administration of Equron. Some random variations in specific parameters in each of these three test areas were present during the pre-treatment acclimation period, but in general all experimental animals were considered normal prior to beginning the study. Random variations persisted throughout the study, but these variations were

consistently greater between animals than those observed for any individual animal. Values outside the normal range during a specific testing period for one laboratory were usually within the normal range for the other laboratory or within range of those published by Veterinary Values. If this was not the case, the animal usually returned to normal by the next testing period. No trends in any of the laboratory analyses were observed which would inidcate systemic toxicity. At the completion of the study all animals were judged normal in this respect.

The results of the physical examinations conducted throughout the study were consistent with the results of the hematology, blood and serum chemistries and urinalyses. No changes in overall physical condition were observed which would suggest systemic toxicity arising from the chronic sodium hyaluronate administration.

Post-injection effusions were observed in the tibiotarsal joints soon after the study began. These were characterized as a diffuse enlargement of the joint capsule noticeable within 24 hours after injection. The degree of enlargement was consistently greater in the hyaluronate treated side. Any swelling observed in the saline control joints subsided rapidly within 24 hours after development. Resolution of the swelling in the treated side took somewhat longer, requiring approximately 48 hours to return to normal. These swellings were not accompanied generally by any heat or lameness. In a few random cases, any lameness present was quickly resolved within 48 hours of injection. The radiocarpal joints remained unremarkable. These tarsal effusions were most likely caused by the large volume of Equron injected and were not considered evidence for inflammatory response. At the end of the study, all joint capsules exhibited some degree of thickening as a result of the repeated arthrocentesis.

Similar to the clinical examinations, the sequential synovial fluid analyses gave no evidence of intra-articular inflammation over the treatment period. Minor changes in synovial fluid properties were observed as in the acute toxicology study. As illustrated in Figures 5 and 6 below for the tibiotarsal joints, the protein concentration and relative viscosity of the Equron and control joints varied randomly over the course of the study. Mean ln relative viscosities of the control joints were slightly higher than the Equron treated joints although there was considerable overlap between the two groups. Mean protein concentration was slightly elevated in the treated group relative to the controls throughout the study. These differences were not considered indicative of significant intra-articular inflammation. Similar results were observed in the carpal joints. The remaining synovial fluid parameters, hyaluronate concentration, mucin clot quality, and WBC content showed some random variations but remained essentially within normal limits throughout the study.

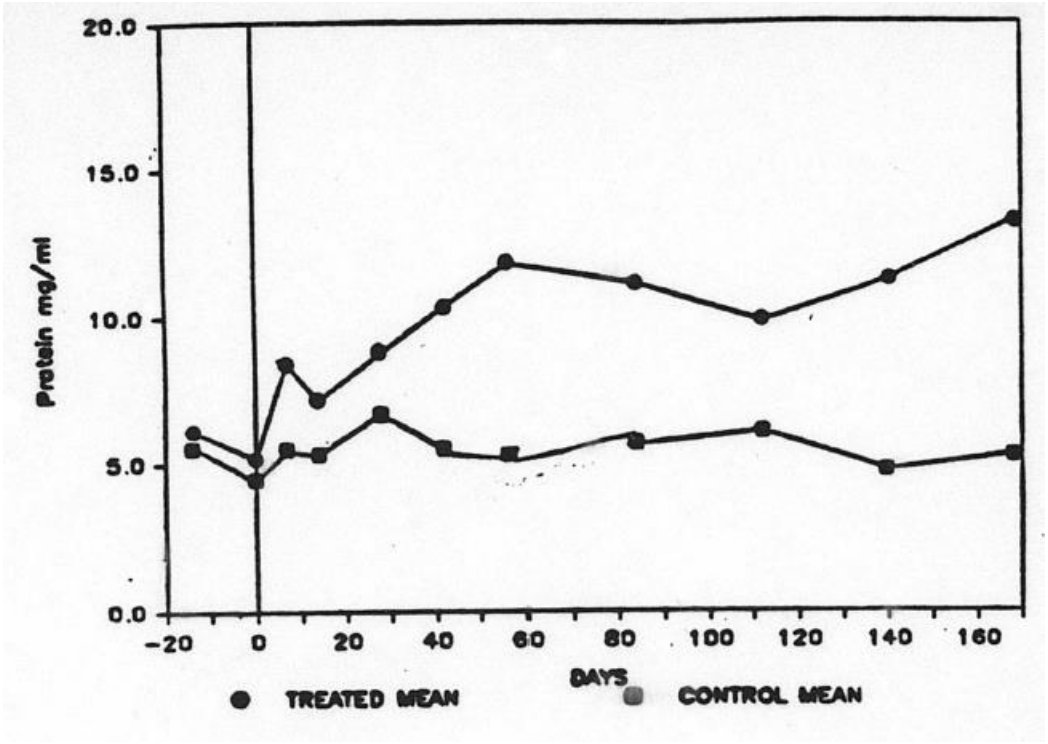

FIGURE 5: CHRONIC TOXICITY: MEANS FOR TREATED AND CONTROL TIBIOTARSAL SYNOVIAL FLUID PROTEIN CONCENTRATION.

Plotted are the means for tibiotarsus synovial fluid protein concentration in mg/ml on the days indicated during the chronic toxicity study of Equron. The data shown for days -14, and 0 represent pretreatment values. Twelve Equron injections (80 mg/tibiotarsus) were given at 14 day intervals immediately following synovial fluid sampling beginning on day 0. The control joint received equivalent volumes of sterile, pyrogen-free saline. The means in each group are comprised of 6 values from six horses. Standard deviation error bars were omitted for clarity. There was, however, considerable overlap between the two groups.

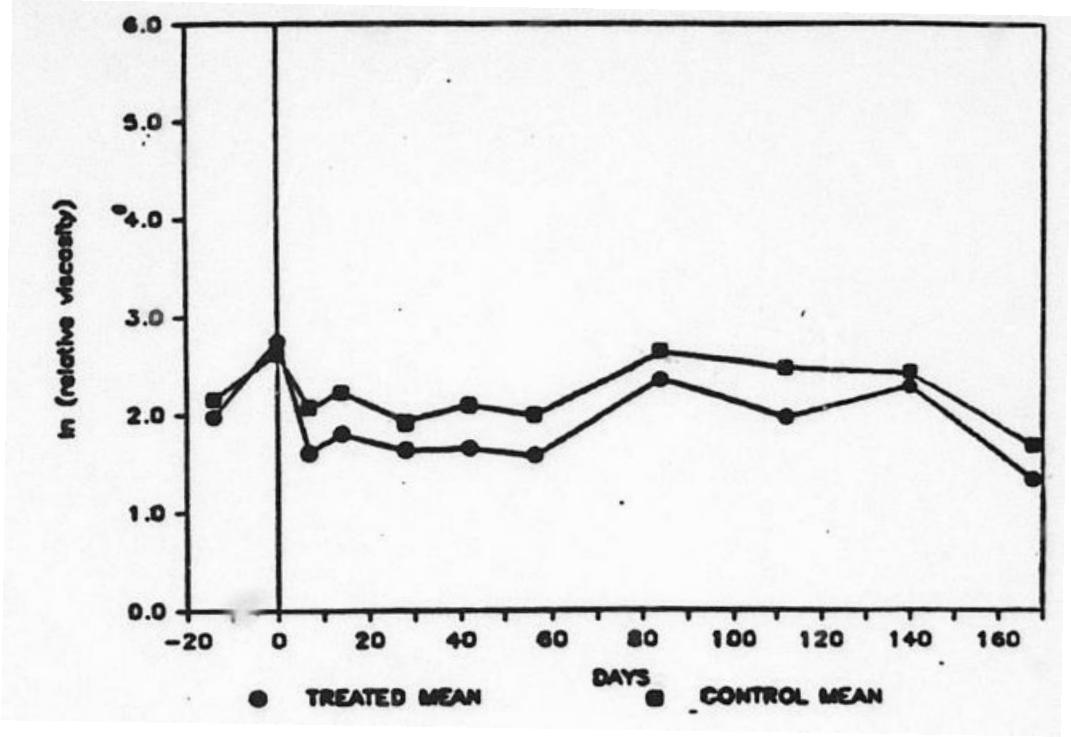

FIGURE 6: CHRONIC TOXICITY: MEANS FOR TREATED AND CONTROL TIBIOTARSAL Ln (RELATIVE VISCOSITY)

The relative viscosity of the synovial fluid from the treated and control tibiotarsal joints of the six horses was measured in centistokes and converted to the natural log for mean calculation and plotting. The data is presented as described in Figure 6.

Gross and histopathological examination of three of the six horses showed no drug related gross or microscopic lesions in any of the organs examined. The radiocarpal and tibiotarsal joints were examined thoroughly both grossly and microscopically. None of the joints showed evidence of inflammation in the internal structures and substances. Synovial fluid was normal in quantity and quality the articular cartilage and subchondral bone were normal as were the ligamentous structures.

The peri-articular tissues and synovial membranes of the tibiotarsal joints exibited some changes suggestive of reactive fibroplasia. These changes were evident in the saline treated joints but were more pronounced in the HA treated joints. From the description and comments of the pathologist, it is evident that some of this reaction was associated with the chronic distention observed largely in the study by the clinical veterinarians, as well as to the repeated trauma of needle penetration for multiple injections and arthrocenteses which contributed to inflammatory edema and healing fibrosis.

The three ramaining animals were held for further evaluation to determine if the fibroplasia of the peri-articular tissues of the tibiotarsal joint would

resolve once arthrocentesis was ceased. Arthroscopic examination and tissue biopsy of the Equron treated joints showed a marked resolution of the fibroplasia over a three month period. By the end of this period the joints were essentially normal.

c. Statistical analysis

Since the observations were similar to those in the acute toxicity study and overall unremarkable, statistical analysis was not considered appropriate.

d. Adverse reactions

No evidence of any systemic adverse reaction was observed in any of the experimental animals. The localized diffused swelling noted in the tibiotarsus appeared to be related to repeated arthrocentesis and the osmotic effects of large volumes of Equron rather than any adverse reaction to the product itself.

e. Conclusion

The results of this study indicated that Equron does not produce systemic adverse effects over long term chronic administration. Repeated injections of the tibiotarsus with large volumes (10 ml) and the resulting chronic distention of the joint may contribute to fibroplasia of the joint capsule. This condition is reversible once arthrocentesis is ceased.

IV. HUMAN FOOD SAFETY

Data on human safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. This drug is approved for use only in horses that are not to be used for food and is to be labeled:

Warning: Not for use in horses intended for food.

Human safety relative to possession, handling and administration:

Hyaluronate sodium is a naturally occurring substance in the connective tissue and synovial fluid of both man and animals. As such, there are no special handling requirements for this drug. As a prescription drug, there are adequate directions in the labeling for the proper use of Equron Injection by the veterinarian.

V. AGENCY CONCLUSIONS

The data submitted in support of this New Animal Drug Application comply with the requirements of Section 512 of the Act 514.11 of the implementing regulations. It demonstrates that Equron (hyaluronate sodium), when used under its labeled conditions of use, is safe and effective.

The safe and effective use of Equron (hyaluronate sodium) injected intra-articularly in horses requires a surgical technique and knowledge of the anatomy of the specific joint. Thus, the expertise of a veterinarian is necessary for safe and effective administration. Accordingly, labeling for Equron must bear veterinary prescription legend.

1. Appendix - List of names and addresses of investigators participating in toxicology, dose determination, and field evaluaion of Equron in the U.S..

VI. ATTACHMENTS

Equron® product label
Equron® packge label
Equron® syringe label

Copies of the attachment and labels may be obtained by writing to the:

Food and Drug Administration
Freedom of Information Staff (HFI-35)
5600 Fishers Lane
Rockville, MD 20857

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

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.