FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 101-479

BANAMINE® Injectable Solution
(flunixin meglumine)

"... indicated for the control of pyrexia associated with bovine respiratory disease and endotoxemia and control of inflammation in endotoxemia in beef and nonlactating dairy cattle."

Sponsored by:

SCHERING-PLOUGH ANIMAL HEALTH

Date Approved: May 5, 1998

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FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

NADA Number: 101-479

Sponsor: Schering-Plough Animal Health Corporation
1095 Morris Ave.
Union, New Jersey 07083-1982

Established Name: flunixin meglumine

Tradename: BANAMINE® Injectable Solution - 50 mg/mL

Marketing Status: This is a prescription product and will include the caution statement as follows: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Supplement Effect: This supplement provides for an additional species, cattle, to be added to the previously approved product, BANAMINE® Injectable Solution.

II. INDICATIONS FOR USE

BANAMINE® Injectable Solution is indicated for the control of pyrexia associated with bovine respiratory disease and endotoxemia. BANAMINE® Injectable Solution is also indicated for the control of inflammation in endotoxemia.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND RECOMMENDED DOSAGE

A. *Dosage Form*: BANAMINE® Injectable Solution is a sterile solution available in 50 mL, 100 mL and 250 mL multidose vials. Each milliliter contains 50 mg flunixin as the meglumine salt.

BANAMINE® Injectable Solution should be stored at controlled room temperature (2 to 30 ºC or 36 to 86 ºF).

B. *Route of Administration*: Intravenous injection

C. *Approved Dose*: The recommended cattle dose is 1.1 to 2.2 mg/kg (1 to 2 mL per 100 lbs) of bodyweight given by slow intravenous administration either once a day as a single dose or divided into two doses administered at 12-hour intervals for up to three days. The total daily dose should not exceed 2.2 mg/kg of bodyweight. Avoid rapid intravenous administration of the drug.

IV. EFFECTIVENESS

Data from the following two dose determination studies and clinical field trials conducted in various geographic locations demonstrate that BANAMINE® Injectable Solution is effective for the reduction of inflammatory eicosanoids in a model of inflammation, reduction of fever in an endotoxemia model, and reduction of fever when used as adjunctive therapy in the treatment of bovine respiratory disease.

A. Endotoxin Dose Titration Study

1. Type of Study: Dose response study using an *E. coli* endotoxin-induced fever model.

2. Investigator: Donald G. Campbell
Schering Corporation
Animal Health Research Center
Allentown, NJ 08501

3. General Design:

a. Purpose: To establish the effective dose of flunixin for fever utilizing an *E. coli* endotoxin-induced fever model.

b. Animals: 67 Hereford heifers ranging in weight from 100-300 kg. Calves ranged in age from 10 to 14 months.

c. Control: Negative control was a non-drug placebo administered intravenously at 1 mL per 22.7 kg body weight, single injection.

d. Study Design: There were 4 treatment groups with 16 calves in the control group, 18 animals in the low BANAMINE® dose group, 16 animals in the middle BANAMINE® dose group, and 17 animals in the high BANAMINE® dose group. Calves received an intravenous injection of *E. coli* endotoxin which induced significant temperature elevations which peaked at 4 hours and then gradually declined. Temperatures were recorded prior to endotoxin injection and at 1, 2, 3, 4, 5, and 6 hours after endotoxin injection. After the 1 hour post-endotoxin injection temperature reading, the BANAMINE® and control treatments were administered.

e. Test Article: Injectable solution containing 50 mg flunixin per mL.

f. Route of Administration: Intravenous injection

g. Dose: Doses were 0, 0.22, 2.2, and 6.6 mg/kg body weight administered as a single dose.

h. Test Duration: 6 hours

i. Pertinent Parameters Measured: The body temperature results were compared for each of the four treatment groups at each time interval.

4. Results:

Treatment with 0.22 mg/kg bodyweight of flunixin resulted in a lower mean temperature than the negative control group at one hour post-treatment, but temperatures were higher than the negative control group thereafter.

Animals receiving the 2.2 mg/kg bodyweight dose did not show substantial increases in temperature after treatment. The 2-, 3-, and 4-hour temperatures remained within 0.3 °F of the reading at the time of treatment (1 hour). Temperature differences between the negative control group and the 2.2 mg/kg group were statistically significant at 2, 3, 4, and 5 hours.

Animals given 6.6 mg/kg behaved much the same as those given 2.2 mg/kg, as their temperatures remained near the 1 hour value at 2, 3, and 4 hours. Temperatures in the 6.6 mg/kg group declined to lower than pre-endotoxin levels at 5 and 6 hours. Differences between 2.2 and 6.6 mg/kg were significant only at 5 and 6 hours.Results are summarized in Table 4.1.

Table 4.1. Mean body temperature (ºF) and statistical analysis for cattle in an *E. coli* induced endotoxin model study.

Treatment group	Pre-tx	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Control	101.7	102.7	103.5	104.3	104.3	103.3	102.6
0.22 mg/kg	101.6	103.0	103.1	105.2	105.3	104.2	102.9
2.2 mg/kg	101.8	102.9	102.7	102.7	103.0	102.4	102.3
6.6 mg/kg	101.9	103.0	102.8	102.4	102.5	101.7	101.7
Comparisons/ p-values							
2.2 mg vs control		p=0.398	p=0.007	p<0.001	p=0.002	p=0.003	p=0.387
2.2 mg vs 0.22 mg		p=0.789	p=0.161	p<0.001	p<0.001	p<0.001	p=0.026
2.2 mg vs 6.6 mg		p=0.739	p=0.538	p=0.482	p=0.231	p=0.009	p=0.012
0.22 mg vs control		p=0.259	p=0.140	p=0.051	p=0.044	p<0.001	p=0.188
0.22 mg vs 6.6 mg		p=0.942	p=0.427	p<0.001	p<0.001	p<0.001	p<0.001
6.6 mg vs control		p=0.240	p=0.031	p<0.001	p<0.001	p<0.001	p<0.001

5. Statistical Analysis:

   Two way analysis of variance was performed on the temperature readings by extracting the treatment and segment effects and the interaction. Pairwise comparisons were made.

6. Conclusions:

   This study demonstrated the antipyretic effect of flunixin in the treatment of *E. coli* endotoxin-induced fever. The study demonstrated that a dose of 2.2 mg/kg was effective in preventing the rise in temperature generated by the administration of endotoxin.

7. Adverse Reactions: No adverse reactions were noted in any treatment group.

B. Dose Titration Study

1. Type of Study: Dose-response relationships for flunixin in a bovine model of acute non-immune inflammation

2. Investigator: Professor Peter Lees
   Dr. D. White
   Royal Veterinary College
   Hatfield, Herts AL9 7TA, U.K.

3. General Design:

   a. Purpose: To study the effect of BANAMINE® Injectable Solution on eicosanoid synthesis in serum and exudate using a model of acute non-immune inflammation.

   b. Animals: 24 weaned beef calves weighing between 57.5 and 93.0 kg.

   c. Controls: Negative control (placebo vehicle)

   d. Study Design: There were 6 treatment groups with 4 calves per group. Inflammation was induced through the implantation of carrageenan soaked sponges into surgically dissected subcutaneous pouches in the neck. The first set of five sponges was implanted at the time of treatment. Sponges were then removed at 1, 2, 4, 6, and 8 hours post-treatment. Five additional sponges were then inserted in each pouch and were subsequently harvested at 10, 12, 15, 24 and 36 hours.

   e. Test Article: The dosage form was an injectable solution containing 50 mg flunixin per milliliter as the meglumine salt.

f. Route of Administration: Intramuscular injection

g. Doses: 0, 0.081, 0.24, 1.1, 2.2, and 6.6 mg/kg bodyweight administered once.

h. Test Duration: 36 hours post treatment

i. Pertinent Parameters Measured: Exudate was harvested from the sponges at selected time points post-treatment (1, 2, 4, 6, 8, 10, 15, 24, and 36 hours) and subjected to eicosanoid analyses (TXB_2 and PGE_2) and measurement of total leukocyte counts. Blood samples were taken at these time points for determination of flunixin plasma levels and serum thromboxane B_2. Swelling of the pouches was also assessed at each time point.

4. Results:

Flunixin did not significantly affect white blood cell numbers in the inflammatory exudate. Lesion swelling was not affected by any of the doses of flunixin administered in the study when lesion volumes were compared to those of the placebo group.

At early sampling times, a high level of inhibition of prostaglandin (PGE_2) and thromboxane (TXB_2) occurred in the higher dose groups (1.1, 2.2 and 6.6 mg/kg bodyweight). A relationship between the administered dose and percent inhibition of PGE_2 and TXB_2 in exudate and TXB_2 in serum became apparent for the three higher dose rates at sampling times of 10 hours and later. The percent inhibition was generally greater with the 2.2 mg/kg dose than with 1.1 mg/kg, particularly from 12 to 24 hours. The inhibition obtained with the 6.6 mg/kg dose was either similar or slightly greater up to 24 hours post-dosing in comparison to the 2.2 mg/kg dose. Results are summarized in Table 4.2.

Table 4.2. Percent inhibition of serum and exudate TXB_2 and exudate PGE_2 relative to control.

Percent Inhibition of Serum TXB_2

flunixin mg/kg	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	15 hr	24 hr	36 hr
0.081	73.7	62.1	42.1	5.8	-18.2	-12.4	-26.2	-2.2	-39.8	-40.2
0.24	97.7	92.2	91.8	68.8	71.3	56.1	51.8	44.9	35.2	17.0
1.10	99.0	95.8	90.8	94.3	96.1	79.1	59.6	44.4	10.7	-43.9
2.20	99.6	98.7	99.2	98.9	99.3	96.7	97.0	92.8	82.6	17.4
6.60	99.8	99.8	99.7	99.7	99.7	99.2	99.0	98.0	94.3	69.5

Percent Inhibition of Exudate PGE_2

flunixin mg/kg	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	15 hr	24 hr	36 hr
0.081	-40	53	49	45	39	25	28	-16	-1	-36
0.24	-8	60	52	45	52	43	21	-47	16	-40
1.10	-5	52	77	95	92	88	89	67	36	-12
2.20	-5	57	89	94	94	92	91	85	56	9
6.60	8	50	86	97	98	95	95	97	86	59

Percent Inhibition of Exudate TXB_2

flunixin mg/kg	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	15 hr	24 hr	36 hr
0.081	-65	-67	6	18	38	30	7	2	-28	-25
0.24	13	45	58	77	86	67	53	57	45	40
1.10	37	35	74	92	95	93	84	76	16	56
2.20	2	10	73	92	96	95	95	96	71	64
6.60	45	64	87	92	96	96	97	96	69	54

Flunixin absorption following intramuscular injection was rapid in all flunixin treated animals. T_{max} was generally 15 minutes or less.

5. Conclusions: Similar efficacy is likely from 0 to 12 hours with the 1.1 or 2.2 mg/kg doses, however a more prolonged action could be expected with the 2.2 mg/kg dose. The dose of 2.2 mg/kg bodyweight produced greater inhibition of PGE_2 and TXB_2 than the 1.1 mg/kg bodyweight dose during the 12 to 24 hour post-dosing period. Greater inhibition with the 6.6 mg/kg dose was generally apparent only during the 24- to 36-hour time period.

C. Field Investigations - Beef Cattle

1. Type of Study:

   Field trials were conducted at 3 locations, in cattle with spontaneously occurring bovine respiratory disease (BRD).

2. Investigators

   E. D. Johnson, D.V.M.
   Johnson Research
   Parma, ID

   M. I. Wray, Ph.D.
   Horton Feedlot and Research Center
   Wellington, CO

   D. P. Hutcheson, Ph.D.
   Texas A & M University
   Amarillo, TX

3. General Design:

   a. Purpose: To evaluate the efficacy and safety of flunixin in conjunction with oxytetracycline antibiotic in the treatment of naturally occurring bovine respiratory disease as compared to oxytetracycline antibiotic treatment alone.

   b. Animals: 363 calves (heifers, steers and bulls) ranging from 6 to 12 months of age with a mean weight of 420.4 lbs.

   c. Controls: The control product was an approved oxytetracycline injectable solution administered for 3 consecutive days at the dose of 10 mg oxytetracycline/kg body weight (4.5 mg/lb)

   d. Diagnosis: The diagnosis of naturally-occurring bovine respiratory disease was based on acute clinical signs of pneumonia with an elevated rectal

temperature (104°F or greater) and respiratory rate (40 per minute or greater). Pretrial nasal swabs were taken for bacterial examination.

e. Dosage Form , Route of Administration, Dose:

Treatment Group	Dosage Regimen	Route of Administration	Number of Animals
Oxytetracycline plus Banamine Injectable Solution	10 mg/kg SID X 3 days 2.2 mg/kg SID X 1-3 days	Intramuscular Intravenous	181
Oxytetracycline	10 mg/kg SID X 3 days	Intramuscular	182

f. Test Article: BANAMINE® Injectable Solution containing 50 mg flunixin per milliliter as the meglumine salt.

g. Study Design: Calves exhibiting acute clinical signs of pneumonia were selected, ranked by temperature, then randomly assigned to one of two treatment groups:

Group A administered 2.2 mg/kg of flunixin IV for 1 to 3 days and 10 mg/kg of oxytetracycline IM for 3 days, or
Group B administered 10 mg/kg of oxytetracycline IM for 3 days.

The calves in Group A with temperatures below 104°F after the first flunixin treatment did not receive any further flunixin. A second flunixin treatment was given on the second day of treatment to the calves with body temperatures over 104°F. The third flunixin treatment was administered to calves with temperatures over 104°F on the third day of treatment.

h. Test Duration: 10 days (3 days of antibiotic treatment with concurrent flunixin therapy for 1 to 3 days depending on temperature after the first day of treatment and 7 days of post-treatment observation and evaluation).

i. Pertinent Parameters Measured: Rectal temperature, character of respiration (normal, mild/moderate, severe), depression (yes or no), and illness index scores (0=normal, 1=slightly ill, 2=moderately ill, 3=very ill, and 4=moribund). Treatment failure was defined as an animal developing severe recurrent respiratory disease as evidenced by an illness index of 3 or more

from the end of the 3 days of treatment to the end of the study. Mortality, weight gain, daily feed intake, and lung pathology were also evaluated.

4. Results:

Number of flunixin treatments: 61% of the calves in the oxytetracycline plus flunixin group required only one injection of flunixin, 18% required a second treatment with flunixin and 21% required a third flunixin treatment.

Mortality: Fifteen animals in the group receiving oxytetracycline alone died during the course of the study versus eight in the group receiving both oxytetracycline and flunixin. One of the study sites had one mortality in the oxytetracycline group and another site had one mortality in the oxytetracycline and flunixin group. The third site had a concurrent BVD outbreak. At this site, there were seven deaths in the flunixin and oxytetracycline group and fourteen in the oxytetracycline group.

Character of Respiration: At pretreatment (Day 0) and on Day 1, the two groups did not differ significantly with respect to their character of respiration. The character of respiration scores for the flunixin plus oxytetracycline group were better than the corresponding scores for the oxytetracycline group on Day 2 and Day 4. By Day 9, (99/144), 69% of the animals in the flunixin plus oxytetracycline group had their character of respiration return to normal compared to 50% (70/139) in the oxytetracycline group.

Illness Index Scores: Overall, more animals that received flunixin plus oxytetracycline were scored as normal between Day 1 and Day 9 than in the oxytetracycline alone group.

Rectal Temperature: The two treatment groups were comparable with respect to their pretreatment (Day 0) rectal temperatures with both groups having a mean temperature of 104.9 ºF. By Day 1 both treatment groups showed a decrease in temperature with a greater decrease in the group that received flunixin plus oxytetracycline compared to the decrease in the group that received only oxytetracycline. The difference between the two treatment groups was statistically significant on Day 1.

Depression: Fewer animals in the flunixin plus oxytetracycline group showed evidence of depression compared to the group receiving oxytetracycline alone.

Treatment Success/Failure: Twenty-two % of the animals in the flunixin plus oxytetracycline group were treatment failures compared to 26% in the oxytetracycline alone group. Thirty-four % treatment failures occurred on Day 3 in the oxytetracycline alone group, compared to zero in the flunixin plus oxytetracycline group. On Day 4, the corresponding percentages were 13% for

the group receiving oxytetracycline alone and 10% for the flunixin plus oxytetracycline group.

Lung Pathology: Little or no difference in lung pathology was observed between the two treatment groups.

Weight Gain and Daily Feed Intake: Overall the oxytetracycline group gained more weight (16.5 lb vs. 10.6 lb respectively), but the difference was not statistically significant. Little or no difference in terms of daily feed intake was observed between the two treatment groups.

5. Statistical Analysis:

Quantitative variables (temperature, respiration, and weight) were analyzed for within and between treatment group differences with respect to the changes from pretreatment by analysis of covariance, using pretreatment values as the covariate. For the ordered categorical qualitative variables (illness score and character of respiration), the exact p-values of the Wilcoxon Midrank Test were computed. The qualitative variables with only two response categories (depression, mortality, treatment success/failure) were analyzed by a one-tailed Fisher's exact test. The results of all statistical tests were declared significant at the alpha=.05 level.

6. Conclusions:

Flunixin ameliorated some of the clinical signs of bovine respiratory disease and demonstrated efficacy as an antipyretic.

7. Adverse Reactions:

No adverse reactions were reported in either of the treatment groups.

D. Field Investigations - Calves

1. Type of Study:

A field trial was conducted in young Holstein calves with naturally-occurring bovine respiratory disease (BRD).

2. Investigator

   J. Davidson, DVM
   Health Management Services
   Tulare, California

3. General Design:

   a. Purpose: To evaluate the efficacy and safety of flunixin in conjunction with oxytetracycline in the treatment of naturally-occurring bovine respiratory disease as compared to oxytetracycline treatment alone.

   b. Animals: 81 male Holstein calves ranging from 3-4 months of age with a mean weight of 82.3 kg.

   c. Controls: The control product was an approved oxytetracycline injectable solution administered for 3 consecutive days at the dose of 10 mg oxytetracycline/kg body weight (4.5 mg/lb)

   d. Diagnosis: The diagnosis of naturally-occurring bovine respiratory disease was based on acute clinical signs of pneumonia with an elevated rectal temperature (104 °F or greater) and respiratory rate (40 per minute or greater). Pretrial nasal swabs were taken for bacterial examination.

   e. Dosage Form , Route of Administration, Dose:

Treatment Group	Dosage Regimen	Route of Administration	Number of Animals
Oxytetracycline plus Banamine Injectable Solution	10 mg/kg SID X 3 days 2.2 mg/kg SID X 1-3 days	Intramuscular Intravenous	43
Oxytetracycline	10 mg/kg SID X 3 days	Intramuscular	38

   f. Test Article: Banamine Injectable Solution contains 50 mg flunixin per milliliter as the meglumine salt.

   g. Study Design: Calves exhibiting acute clinical signs of pneumonia were selected, ranked by temperature, then randomly assigned to one of two treatment groups:

Group A administered 2.2 mg/kg of flunixin IV for 1 to 3 days and 10 mg/kg of oxytetracycline IM for 3 days, or

Group B administered 10 mg/kg of oxytetracycline IM for 3 days.

The calves in Group A with temperatures below 104 °F after the first flunixin treatment did not receive any further flunixin. A second flunixin treatment was given on the second day of treatment to the calves with body temperatures over 104 °F. The third flunixin treatment was administered to calves with temperatures over 104 °F on the third day of treatment.

h. Test Duration: 10 days (3 days of antibiotic treatment with concurrent flunixin therapy of 1 to 3 days depending on temperature after the first day of treatment and 7 days of post-treatment observation and evaluation).

i. Pertinent Parameters Measured: Rectal temperature, character of respiration (normal, mild/moderate, severe), depression (yes or no), and illness index scores (0=normal, 1=slightly ill, 2=moderately ill, 3=very ill, and 4=moribund). Treatment failure was defined as an animal developing severe recurrent respiratory disease as evidenced by an illness index of 3 or more from the end of the 3 days of treatment to the end of the study. Mortality, weight gain, daily feed intake, and lung pathology were also evaluated.

4. Results:

Number of flunixin treatments: 58.1% of the calves in the oxytetracycline plus flunixin group required only one injection of flunixin, 34.9% required a second treatment with flunixin and 7% required a third flunixin treatment.

Mortality: No deaths were recorded during this study

Character of Respiration: The character of respiration was normal on Day 0 (pre-treatment) for 9/43 (21%) of the animals in the flunixin plus oxytetracycline group and for 12/38 (32%) of the animals in the oxytetracycline alone group. Between Day 2 and Day 7 more animals in the flunixin plus oxytetracycline group had normal character of respiration than in the oxytetracycline alone group.

Illness Index Scores: A statistically significantly greater number of animals were scored as normal on Day 2 and Day 3 in the flunixin plus oxytetracycline group than in the oxytetracycline alone group.

Rectal Temperature: On Day 0, the flunixin plus oxytetracycline group had a mean temperature of 104.9 ºF and the oxytetracycline alone group had a mean temperature of 104.6 ºF. By Day 1 both treatment groups showed a decrease in temperature with a greater decrease in the group that received flunixin plus

oxytetracycline compared to the decrease in the group that received only oxytetracycline. The differences between the two treatment groups with respect to their mean changes in temperature achieved statistical significance on Days 1, 2, and 3.

Depression: Calves receiving both flunixin plus oxytetracycline showed consistent improvement in demeanor from Day 2 to Day 7 as compared to the oxytetracycline alone group.

Treatment Success/Failure: Forty % of the animals in the flunixin plus oxytetracycline group were treatment failures compared to 47% in the oxytetracycline alone group. Forty-four % of the treatment failures occurred on Day 3 in the oxytetracycline alone group, compared to 24% in the flunixin plus oxytetracycline group.

Weight gain: Calves receiving flunixin and oxytetracycline gained slightly more weight than the oxytetracycline group over the study (4.6 kg vs 4.0 kg, respectively).

5. Statistical Analysis:

Quantitative variables (temperature, respiration, and weight) were analyzed for within and between treatment group differences with respect to the changes from pretreatment by analysis of covariance, using pretreatment values as the covariate. For the ordered categorical qualitative variables (illness score and character of respiration), the exact p-values of the Wilcoxon Midrank Test were computed. The qualitative variables with only two response categories (depression, mortality, treatment success/failure) were analyzed by a one-tailed Fisher's exact test. The results of all statistical tests were declared significant at the alpha = .05 level.

6. Conclusions:

Flunixin ameliorated some of the clinical signs of bovine respiratory disease and demonstrated efficacy as an antipyretic.

7. Adverse Reactions:

One adverse reaction was reported during the early phase of the study in which one animal receiving product suffered a knock-down effect due to rapid IV administration. The investigator, thereafter, administered the drug slowly without further signs re-appearing.

E. Published Literature Supporting Endotoxemia and Lower End of Dose Range (1.1 mg/kg)

A model of non-immune acute inflammation developed in horses, [Higgens, et al.][1] has been adapted for use in cattle, and is the basis of the flunixin dose titration study by Lees, P. and White, D. referred to in section B above. The study in cattle shows the dose-dependent effect of flunixin and demonstrates that flunixin produces a reduction or abolition of the production of serum thromboxane (TXB_2), exudate TXB_2, and prostaglandin (PGE_2). A direct relationship between administered dose and the percentage of inhibition was apparent for the higher dose rate sampling times of 10 hours and later. Percentage inhibition was greater with a 2.2 mg/kg dose than with 1.1 mg/kg, particularly from 12 to 24 hours. Whereas similar clinical efficacy is likely to occur from 0 to 12 hours (for the 1.1 and 2.2 mg/kg doses), a more prolonged (greater than 12 hours) clinical action would be expected with the 2.2 mg/kg dose.

A pharmacodynamic study [Landoni, et al. (Ref 2)] was conducted to relate plasma pharmacokinetics to the ability of NSAIDs to inhibit the production of mediators (inflammatory eicosanoids) of the cyclooxygenase pathway (TXB_2 in serum/exudate and PGE_2 in inflammatory exudate and transudate). The study demonstrated that flunixin penetrated very poorly into interstitial fluid (due to the very high degree of binding to plasma protein), but readily penetrated into and was only slowly cleared from acute inflammatory exudate. This accumulation of drug at sites of inflammation explains why plasma blood levels do not correlate with clinical efficacy.

References 3 through 7 describe studies that demonstrate the efficacy of flunixin at a dose of 1.1 mg/kg body weight, given once or twice at 8 hour intervals, or 2.2 mg/kg given once, as adjunctive therapy in the treatment of endotoxemia in cattle. The studies demonstrate that flunixin reduces the production of the inflammatory eicosanoids that mediate some of the endotoxin-induced changes seen in endotoxemia in neonatal calves and nonlactating dairy cattle. Flunixin is effective in the reduction of clinical signs associated with endotoxemia such as fever and depression in neonatal calves, and rumen stasis in neonatal calves and nonlactating dairy cattle.

References:

1. Higgins, A.J., and Lees, P. (1984). Arachidonic acid metabolites in carrageenan-induced equine inflammatory exudate. *Journal of Veterinary Pharmacology and Therapeutics,* 7:65-72.

2. Landoni, M.F., Cunningham, F.M., and Lees, P. (1995). Determination of pharmacokinetics and pharmacodynamics of flunixin in calves by use of pharmacokinetic/pharmacodynamic modeling. *American Journal of Veterinary Research,* 56:786-794.

3. Semrad, S.D. (1993). Comparison of flunixin, prednisolone, dimethyl sulfoxide, and a lazaroid (U74389F) for treating endotoxemic neonatal calves. *American Journal of Veterinary Research,* 54:1517-22.

4. Semrad, S.D. (1993). Comparative efficacy of flunixin, ketoprofen, and ketorolac for treating endotoxemic neonatal calves. *American Journal of Veterinary Research,* 54:1511-6.

5. Templeton, C.B., Bottoms, G.D., Fessler, J.F., and Turek, J.J. (1988). Hemodynamics, plasma eicosanoid concentrations, and plasma biochemical changes in calves given multiple injections of *Escherichia coli* endotoxin. *American Journal of Veterinary Research,* 49:90-5.

6. Eades, S.C. (1993). Endotoxemia in dairy cattle: role of eicosanoids in reticulorumen stasis. *Journal of Dairy Science,* 76:414-20.

7. Luthman, J., Kindahl H., and Jacobsson, S.O. (1989). The influence of flunixin on the response to *Salmonella typhimurium* endotoxin in calves. *Acta vet. scand.,* 30:295-300.

F. Pharmacokinetics

Flunixin meglumine is a weak acid (pKa = 5.82)[1] which exhibits a high degree of plasma protein binding (approximately 99%)[2] . However, free (unbound) drug appears to readily partition into body tissues [V_{ss} predictions range from 297 to 782 mL/kg[2,3,4,5]. Total body water is approximately equal to 570 mL/kg[6]]. In cattle, elimination occurs primarily through biliary excretion[7] . This may, at least in part, explain the presence of multiple peaks in the blood concentration/time profile following IV administration[2].

Each investigation reported a much smaller value for volume of distribution at steady state (V_{ss}) as compared to that for the volume of distribution associated with the terminal elimination phase (V_β). This disparity appears to be attributable to an extended drug elimination from a deep compartment[8]. The presence of a deep (third) compartment may be observed when drug concentrations fall below 0.1 µg/mL[5]. The disparity is also consistent with a large distribution volume for free drug (bound drug remains in the blood). The presence of a tri-exponential decay curve was reported in calves with induced acute inflammatory reaction[9].

Actual values of body clearance (CL), V_{ss}, V_β and terminal elimination half-life ($Tl/2_\beta$) differed markedly across studies. This discrepancy may, at least in part, have been associated with the handling of terminal flunixin concentrations in the data analysis. Table 4.3 summarizes flunixin pharmacokinetics in cattle as estimated across five investigations.

TABLE 4.3: PHARMACOKINETIC ESTIMATES ACROSS FIVE STUDIES

	Odensvik, and Johansson[2]		Anderson, et al[3]	Odensvik[4]	Hardee, et al.[5]	Landoni, et al.[9]
$V\beta$ (mL/kg)	789[a]	764[b]	684	858	1050	2110
V_{ss} (mL/kg)	419[a]	297[b]	397	782	500	712
$V_{ss}/ V\beta$	0.53[a]	0.39[b]	0.58	0.91	0.48	0.34
T 1/2 (hr)	3.8[a]	4.3[b]	3.14	5.2	8.12	6.87
Cl (mL/kg/hr)	142[a]	122[b]	151	115	90	200
AUC (µg*hr/mL)	15.5[a]	18.0[b]	15.11	20	24.4	11.8

*AUC (zero to infinity) values normalized to a 2.2 mg/kg dose

a: values obtained in a single primiparous cow (2 compartment open body model).
b: values obtained in one heifer (2 compartment open body model).
3: values obtained in twelve lactating Holstein cows (2 compartment open body model).
4: values obtained in six heifers, 407 to 562 kg (noncompartmental analysis).
5: values obtained in six non-lactating Holstein-Friesian heifers, 346 to 506 kg (2 compartment open body model).
9: values obtained in Friesian-type calves (approximately 120 kg) with carrageenan-induced inflammatory reaction (3 compartment open body model).

The pharmacokinetic estimates reported by Landoni, *et al*[9] differ from those of the other four investigations [2,3,4,5]. This may be attributable to induction of an inflammatory reaction in these study subjects. Landoni, *et al* reported exudate $AUC_{O\text{-}inf}$ values which are twice that observed in plasma (i.e., 11.81 µg*hr/mL in plasma vs 27.57 µg*hr/mL in the exudate). This suggests sequestering of drug at the site of the infection. If the drug remained within the exudate for prolonged periods and returned slowly to the blood (resulting in flunixin plasma concentrations

near the assay limit of quantification), these terminal drug concentrations may have been excluded from the estimates of plasma $AUC_{O\text{-}inf}$. Such exclusion would explain the unusually low plasma AUC value reported in this study. Concomitantly, it would result in an increase of the estimates of CL and $V\beta$, when calculated as dose/$AUC_{O\text{-}inf}$ and dose/AUC^*_β respectively. A very slow elimination process is consistent with the 11.62 hours mean T_{MAX} value for flunixin in exudate (2 calves exhibiting exudate C_{MAX} values at 24 and 30 hours after IV administration).

Flunixin persists in inflammatory tissues[10] and is associated with anti-inflammatory properties which extend well beyond the period associated with detectable plasma drug concentrations [4,10]. These observations account for the counterclockwise hysteresis associated with flunixin's pharmacokinetic/pharmacodynamic relationships [9]. Therefore, prediction of drug concentrations based upon the estimated solution plasma terminal elimination half-life will likely underestimate both the duration of drug action and the concentration of drug remaining at the site of activity.

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V. ANIMAL SAFETY

A Target Animal Safety Study in beef calves was conducted to address the safety of multiple injections of BANAMINE® Injectable Solution at 1X (2.2 mg/kg), 3X (6.6 mg/kg), and 5X (11.0 mg/kg) dose levels (based on the high end of the dose range). An Acute Toxicity Study in young beef cattle was conducted to determine the tolerance to and the clinical profile of 3X, 6X, 12X, and 24X overdoses of the high end of the intended clinical dose range of BANAMINE® Injectable Solution. Two Reproductive Safety Studies in cows were performed to determine if there were any adverse effects on breeding cows.

A. Target Animal Safety (1X, 3X, 5X)

1) Type of Study: This was a 10 day study in which a total of nine intravenous injections of BANAMINE® Injectable Solution at doses of 0, 2.2, 6.6, and 11 mg of flunixin/kg of body weight were administered to Hereford beef calves.

2) Study Director: Dan Ronning
Colorado Animal Health Research Enterprises
Fort Collins, Colorado

3) General Design:

a. Purpose: To characterize the responses of beef cattle to intravenous administration of flunixin at doses of 0X, 1X, 3X, and 5X of the 2.2 mg/kg intended dose.

b. Animals: Twenty-four (24) Hereford beef calves (12 male and 12 female), approximately 5-6 month of age and weighing 114-219 kg at the initiation of dosing.

c. Control: Normal physiologic saline

d. Test Article: Injectable solution containing 50 mg of flunixin/mL.

e. Route of Administration: Intravenous Injection

f. Doses: 0, 2.2, 6.6,and 11.0 mg/kg body weight daily for 9 days.

g. Test Duration: 10 days

h. Pertinent Parameters/Observations: Clinical observations, veterinary conducted physical examinations, feed and water consumption, body weights, hematology, serum chemistry, urinalysis, fecal examinations, gross pathology, and histopathology. Clinical pathology analyses were conducted on Days -3, 0, 3, and 9 (Day 0 being the first day of treatment).

4) Results:

a. Clinical Observations: Clinically-observed fecal blood was the only treatment-related change seen sporadically in the 6.6 and 11 mg/kg dose groups. In the 6.6 mg/kg group, 4 different animals had fecal blood on one day ranging from Day 3 to Day 9. In the 11 mg/kg dose group, 5 animals had at least one day of fecal blood, with only 2 animals exhibiting it for more than one day.

b. Water Consumption: There were no treatment-related effects.

c. Feed Consumption: Slightly decreased feed consumption in the 6.6 and 11.0 mg/kg dose levels were observed.

d. Body Weight: The average daily gain was 0.35 and 0.25 kg for the 0.0 and 2.2 mg/kg dose groups, respectively, and -0.08 and -0.13 kg for the 6.6 and 11 mg/kg dose groups, respectively.

e. Hematology/Serum Chemistry: No treatment-related effects were observed.

f. Urinalysis: Slightly increased incidence of microscopic RBCs and/or occult blood in the 6.6 and 11.0 mg/kg-dosed cattle were observed. One animal in the 6.6 mg/kg group had 2+ urine occult blood on Day 3. In the 11 mg/kg group, one animal had 3+ and one animal had 1+ urine occult blood on Day 3. On Day 9, two different animals in the 11 mg/kg group had 2+ or 1+ urine occult blood. On Day 9, one animal in the 6.6 mg/kg group and one animal in the 11.0 mg/kg group had a moderate number of RBCs in the sediment.

g. Fecal Examinations: Slightly increased incidence of frank and/or occult blood in the 6.6 and 11.0 mg/kg-dosed cattle were observed. One animal in the 6.6 mg/kg group had fecal blood on Days 3 and 9 and another animal in this group had fecal blood on Day 9. In the 11.0 mg/kg group, there was fecal blood in one specimen on Day 3 and in 3 specimens on Day 9.

h. Gross and Histopathological Evaluations: No treatment-related effects were observed.

5) Statistical Analysis: Study data were tabulated and as appropriate summarized through the calculation of mean and standard deviation.

6) Conclusion: Adverse clinical signs of fecal blood and hematuria attributed to flunixin intravenous injections were seen in calves administered elevated doses of 6.6 and 11.0 mg/kg, 3 times and 5 times the high end of the recommended dose range, respectively) for three times the recommended clinical duration. No adverse effects were seen in calves given the high end of the recommended dose range (2.2 mg/kg).

B. Acute Toxicity (3X, 6X, 12X, 24X)

1) Type of Study: This was a 22-day study in which four intravenous injections of BANAMINE® Injectable Solution, administered in sequential doses of 6.6, 13.2, 26.4, and 52.8 mg of flunixin/kg of body weight were administered every other day to crossbred beef calves. There was a 14-day postdose observation period following the last dose.

2) Study Director: Ruta M. Slepytys
Schering Corporation
Animal Health Research Center
Allentown , NJ

3) General Design:

a. Purpose: This study was designed to determine the acute toxicological effects and the acute toxicity of flunixin by intravenous injection to crossbred beef calves.

b. Animals: Four crossbred beef calves (2 males and 2 females) approximately 241-286 kg at initiation of dosing.

c. Control: none

d. Test Article: Injectable solution containing 50 mg flunixin per mL.

e. Route of Administration: Intravenous Injection

f. Doses: 6.6, 13.2, 26.4, and 52.8 mg/kg body weight, administered as sequential doses, every other day for a total of 4 injections.

g. Test Duration: 22 days (includes a 14-day postdose period)

h. Pertinent Measurements/Observations: Clinical observations, survival, hematology, serum chemistries, urinalysis, and fecal blood. Clinical pathology analyses were conducted prior to treatment, 24 hours after each treatment and 24, 48, 72 hours, and 7 and 14 days after the last treatment, Gross and histopathologic evaluation was conducted on the one animal that died during the study.

4) Results

a. Clinical Observations: Petit mal seizures in 1 calf at 26.4 mg/kg and grand mal seizures in all four calves at 52.8 mg/kg.

b. Survival: One calf died following the 52.8 mg/kg dose.

c. Hematology/ Serum Chemistries: Slightly increased platelet numbers were observed.

d. Urinalysis: Hematuria and proteinuria were noted after all doses.

e. Fecal Blood: Frank and/or occult blood was observed after the 13.2 mg/kg, 26.4 mg/kg, and 52.8 mg/kg doses.

f. Gross and Histopathological Observations: No treatment-related changes, only nonspecific congestion or hemorrhages noted in multiple organs of the one animal that died.

5) Statistical Analysis: none

6) Conclusions: Animals at all doses exhibited adverse clinical signs. Blood in the urine and/or feces was seen in animals at all doses (6.6, 13.2, 26.4, and 52.8

mg/kg doses). One animal in the 26.4 mg/kg dose group had a petit mal seizure and all calves in the 52.8 mg/kg dose group had grand mal seizures with one calf dying. In the three remaining animals all parameters returned to normal during the post-dose observation period except for one animal which was negative for frank and occult fecal blood at 7 days but was positive at 14 days, another animal which was negative for urine blood at 7 days but had 12-15 RBCs/HPF, and another animal which was also negative for urine blood at 14 days but had 8-9 RBCs/HPF.

C. Target Animal Reproduction Safety (3X)

1) Type of Study: This was a 12-month reproductive study in which six intravenous injections of BANAMINE® Injectable Solution at 3X (6.6 mg/kg) the recommended high end of the dose range (2.2 mg/kg) were administered to pregnant cattle during selected time points of each trimester of pregnancy.

2) Study Director: James A. Scott
Vetco Research & Development
Great Falls, Montana

3) General Design:

a. Purpose: To determine the effects of overdoses of flunixin on pregnancy in cattle.

b. Animals: Twenty-four crossbred pregnant cattle, approximately 30-36 months of age and weighing 400-535 kg at the time of initiation of dosing.

c. Control: normal physiologic saline

d. Dosage Form: The dosage form was an injectable solution containing 50 mg flunixin per mL.

e. Route of Administration: Intravenous Injection

f. Dose: 6.6 mg/kg body weight (3X).

g. Study Design: Cows were observed for estrus and bred artificially with frozen semen from the same bull. Pregnancy exams were conducted approximately 60 days after breeding. Twenty-four pregnant, healthy cattle were allotted to 2 groups of 12 animals. One group was administered 6.6 mg/kg flunixin and the other was a control group receiving saline. All cattle were treated with two consecutive daily injections once during each trimester of pregnancy, on post-conception Days 91-92, 150-151, and 265-266. The

cows were palpated for maintenance of pregnancy throughout the study on Days 90, 150, and 140 after breeding. The cows were allowed to calve unassisted. Calves were weighed at birth and 30 days after birth. Observations were made of initial suckling behavior and calves were examined for external abnormalities.

h. Test Duration: 12 months

i. Pertinent Parameters/Observations: Data collected on pregnant cows were body weights, pregnancy maintenance, gestation length, and ease of calving. Calf data collected included birth weight, survival and 30-day weight.

4) Results

a. Body Weights: There were no treatment-related effects.

b. Pregnancy Examinations: There were no treatment-related effects.

c. Length of Gestation: All cows in both groups calved. The average gestation length was 281.2 days for the flunixin group and 285.2 days for the saline control.

d. Parturition: All cows calved without assistance.

e. Calf Birth Weights: The average birth weight of calves born to the control animals was 72.2 lb and the average for the flunixin group was 71.9 lb. There were no treatment-related effects at birth.

f. Postnatal Development: One calf in the saline control group died on Day 9 after birth. At Day 30, the calves from treated cows gained slightly more weight than the calves from control cows, 149 lb vs 143.8 lb, respectively.

4) Statistical Analysis

Analysis of covariance (ANCOVA) was used to analyze the body weights of the cattle; the covariant was the body weight at day 91. A two sample t-test was used to analyze the day 91 body weights and the length of gestation. A two-way ANCOVA model was used to analyze the calf birth weights. The efects were sex and treatment, covariate was length of gestation. A two-way ANCOVA model was used to analyze the 30-day calf body weights; the covariants were birth weight and length of gestation. A significance level of 0.05 was used for all comparisons.

5) Conclusion: No adverse effects in the cows or calves were attributed to flunixin intravenous injections administered to the pregnant cows at 6.6 mg/kg, given two times during each trimester.

D. Target Animal Reproduction Safety (3X)

1) Type of Study: This was 13-month study in which twelve intravenous injections of BANAMINE® Injectable Solution at 3X (6.6 mg/kg) the recommended high end of the dose range (2.2 mg/kg) were administered to cattle prior to breeding and during selected time points of pregnancy.

2) Study Director: James A. Scott
Vetco Research & Development
Great Falls, Montana

3) General Design:

a. Purpose: To determine the effects of overdoses of flunixin on breeding and pregnancy in cattle.

b. Animals: Twenty-four crossbred cattle, approximately 30-36 months of age and weighing 380-520 kg at the time of initiation of dosing.

c. Control: normal physiologic saline

d. Test Article: Injectable solution containing 50 mg flunixin per mL.

e. Route of Administration: Intravenous Injection

f. Dose: 6.6 mg/kg bodyweight (3X).

g. Study Design: Twenty-four cows were allotted to 2 groups of 12 animals. One group received 6.6 mg/kg flunixin and the other was a control group receiving saline. The cows were synchronized with 2 doses of prostaglandin F_2 alpha administered 11 days apart. All cows were treated with two consecutive daily injections of flunixin or saline at six time points. The first time was 15/16 days after the second prostaglandin injection. Cows were bred as they came into heat following the second injection. They were treated for the second time 12/13 days after breeding. If they did not return to estrus they were treated in a similar manner at 36/37 days, 112/113 days, 210/224, and 265/266 days post-mating. If a cow did not return to heat after the first breeding, she was rebred and treated on Days 12/13 and subsequently as described. A third return to estrus was treated the same as the second. If a cow returned to estrus a fourth time, she was examined for reproductive soundness.

h. Test Duration: 13 months

i. Pertinent Parameters/Observations: Cows were evaluated for the number of services required for conception. Data collected on pregnant cows were body weights, pregnancy maintenance, gestation length, and ease of calving. Calf data collected included birth weight, survival, and 30-day weight.

4) Results

a. Body Weights: There were no treatment-related effects.

b. Number of Services for Conception: There was one cow in the control group that conceived after 2 breedings. The remainder in the group conceived after one breeding. In the flunixin group there were 3 cows that conceived after 2 breedings, one cow that conceived after 3 breedings, and the remainder conceived after 1 breeding. The service per conception rate was 1.1 for the control and 1.4 for the flunixin group.

c. Pregnancy Examinations: All cows became pregnant and all pregnancies resulted in a live birth.

d. Length of Gestation: The average length of gestation was 282.9 days for the control and 280.3 days for the flunixin group.

e. Parturition: All cows had a calving score of 1 (normal presentation, natural birth, and unassisted delivery).

f. Calf Birth Weights: The birth weights of the calves in the control group averaged 70.6 lb and the average of the flunixin group was 73.1 lb.

g. Postnatal Development: Two calves in the flunixin group died shortly after birth, one from predation and the other from drowning. At Day 30, the

calves from treated cows gained slightly more weight than the calves from control cows, 155.4 lb vs 143.8 lb respectively.

5) Statistical Analysis:

Analysis of covariance (ANCOVA) was used to analyze the body weights of the cattle; the covariant was the body weight at inclusion in the study. A two sample t-test was used to analyze the initial body weights and the length of gestation. A Fisher's Exact Test was used to analyze the number of breeding services required. A two-way ANCOVA was used to analyze the calf birth body weight. A two-way ANCOVA model was used to analyze 30 day body weights; the covariate was birth weight and effects were sex and treatment. A significance level of 0.05 was used for all comparisons.

6) Conclusion: No adverse effects in the cows or calves were attributed to flunixin intravenous injections administered to the cows at 6.6 mg/kg, given once each day for two days at six timepoints: one prebreeding, two shortly after breeding, and one timepoint near the end of each trimester.

VI. HUMAN FOOD SAFETY STUDIES

A. Toxicity Studies

1. 90 Day Oral Toxicity Study in Rats

Report Number P-4575

Study Dates - February 18, 1976 to June 14, 1978

Study Director - E. Schwartz
Schering-Plough, Bloomfield, NJ

Identification of Substance and Dosage Form - an aqueous solution of flunixin meglumine in distilled water.

Species and Strain - Charles River Sprague-Dawley rats

Number of Animals Per Sex Per Treatment Group - 20, the rats were approximately 7 weeks old at the start of dosing.

Drug Levels Tested and Duration of Dosing - 0, 1.5, 3, and 6 mg flunixin free acid/kg/day (equal to 0, 2.5, 5, and 10 mg flunixin meglumine/kg/day) was administered once daily for 13 weeks.

Route of Drug Administration - Oral gavage

Parameters Tested - clinical signs, weekly body weight and food consumption, hematologic, clinical chemistry and urinalysis measurements, selected organ weights, and gross and microscopic pathology.

Significant Toxicity Observed - enlarged mesenteric lymph nodes and intestinal adhesions, thickening and ulceration with associated decreases in hematocrit, hemoglobin and increased leucocyte counts.

No Observed Effect Level - 3.0 mg/kg. This was based on gastrointestinal ulceration and related changes in hematology and enlarged mesenteric lymph nodes observed in rats in the 6 mg/kg dosage group.

2. 90 Day Oral Toxicity Study in Monkeys

Report Number P-4576

Study Dates - March 29, 1976 to June 30, 1978

Study Director - E. Schwartz
Schering-Plough, Bloomfield, NJ

Identification of Substance and Dosage Form - an aqueous solution of flunixin meglumine in distilled water.

Species and Strain - Rhesus monkeys, *Macaca mulatta*

Number of Animals Per Sex Per Treatment Group - 4. The control group consisted of 2 animals per sex.

Drug Levels Tested and Duration of Dosing - 0, 5, 15, 45, 60 mg flunixin free acid/kg/day (equal to 0, 8.3, 24.9, 74.7, and 99.6 mg flunixin meglumine/kg/day) was administered once daily for 13 weeks.

Route of Drug Administration - Oral gavage

Parameters Tested - clinical signs, weekly body weight, food consumption, rectal temperature, respiration rate, heart rate, electrocardiograms, ophthalmic exams, hematology, clinical chemistry and urinalysis measurements, selected organ weights, and gross and microscopic pathology.

Significant Toxicity Observed - decreased body weight gain, hemoglobin, hematocrit, serum total protein and albumin.

No Observed Effect Level - 45.0 mg/kg body weight. This was based on decreased body weight gain, hemoglobin, hematocrit, serum total protein and albumin seen in monkeys in the 60 mg/kg dosage group.

3. One Year Oral Toxicity Study in Rats

Report Number P-5760

Study Date - July 3, 1991 to March 24, 1994

Study Director - Mark Berardi
Schering-Plough, Lafayette, NJ

Identification of Substance - flunixin meglumine

Species and Strain - Charles River, Sprague-Dawley rats

Number of Animals Per Sex Per Treatment Group - 30

Drug Levels Tested and Duration of Dosing - 0, 1.0, 2.0, 6.0 mg of flunixin free acid/kg/day (equal to 0, 1.67, 3.33 and 10 mg flunixin meglumine/kg/day) for 52 weeks.

Route of Drug Administration - oral via dietary admixture

Parameters Tested - Clinical signs, body weight, food and water consumption, ophthalmoscopy, clinical pathology, organ weights, gross and microscopic pathology were evaluated.

Significant Toxicity Observed - decreased body weight gain and serum total protein. Increased incidence: fecal occult blood, gastrointestinal erosions/ulcers, renal papillary necrosis, regenerative anemia and neutrophilia

No Observed Effect Level - 1.0 mg/kg. This was based on decreases in body weight gain and serum total protein observed in males in the 6.0 mg/kg dosage group; increased fecal occult blood, gastrointestinal erosion/ulceration and renal papillary necrosis observed in males in the 2.0 mg/kg and males and females in the 6.0 mg/kg dosage group and regenerative anemia and neutrophilia observed in males and females in the 6.0 mg/kg dosage group.

4. Teratology Study (Segment II) in Rats

Report Number - P-4457

Study Dates - January 18,1976 to January 22, 1977

Study Director- Max F. Klein
Schering-Plough, Lafayette, NJ

Identification of Substance and Dosage Form - an aqueous solution of flunixin meglumine in 2.5 % Tween 80® (controls received only Tween 80)

Species and Strain - Charles River, Sprague-Dawley rats

Number of Animals Per Sex Per Treatment Group - 25 females (controls 35 females)

Drug Levels Tested and Duration of Dosing - 0, 1.5, 3.0. 6.0 mg of flunixin free acid/kg/day (equal to 0, 2.49, 4.98, and 9.96 mg flunixin meglumine/kg/day) on gestation days 6-15.

Route of Drug Administration - Oral gavage

Parameters Tested - maternal toxicity, embryotoxicity, and teratogenicity

Significant Toxicity Observed - moderate maternal toxicity and fetotoxicity

No Observed Effect Level - 3.0 mg/kg. This was based on maternal toxicity (reduced body weight gain , two deaths with gross gastrointestinal lesions) and fetotoxicity (retarded ossification of sternebrae and decreased 24-hour survival). No evidence of treatment-related teratogenicity was noted at any dosage level employed in this study.

5. Genotoxicity Study - DNA Polymerase- Flunixin Meglumine

Report Number D-15338

Study Dates - March 11, 1980 to March 12, 1980

Study Director - Robert W. Naismith
Pharmakon Laboratories, Scranton, PA

Identification of Substance and Dosage Form - Flunixin meglumine powder

Species and Strain - *E. coli* w3110 (pol A^+) and p3478 (pol A^-)

Number of Animals Per Sex Per Treatment Group - not applicable

Drug Levels Tested and Duration of Dosing - 0.03, 0.3, 3, 30 and 300 mg/mL at a volume of 20 µl and 50 µl with and without metabolic activation (S-9 reaction)

Route of Drug Administration - not applicable

Parameters Tested - To determine if a compound is able to modify the DNA of the repair deficient strain of *E. coli* p3478 ($polA^-$) when compared to DNA repair competent strain *E. coli* W3110 ($polA^+$)

Significant Toxicity Observed - Flunixin meglumine produced a significant difference between the zones of inhibition of the DNA repair deficient strain p3478, compared to the DNA repair competent strain W3110 at the 300 mg/mL level.

No Observed Effect Level - not applicable

Statistical Analysis - not applicable

Conclusions - The results of this test indicate that flunixin meglumine can preferentially alter cellular DNA and cause primary DNA damage in the test organism.

6. Genotoxicity Study - DNA Polymerase- Flunixin Meglumine

Report Number - D-15339

Study Dates - January 1982

Study Director - D. Jagannath
Litton Bionetics

Identification of Substance and Dosage Form - Flunixin meglumine powder

Species and Strain - *E. coli* w3110 (pol A^+) and p3478 (pol A^-)

Number of Animals Per Sex Per Treatment Group - not applicable

Drug Levels Tested and Duration of Dosing - 1, 10, 100, 500, 1000, 2500, 5000 and 10,000 µg/plate with and without metabolic activation (S-9 reaction).

Route of Drug Administration - not applicable

Parameters Tested - To determine if a compound is able to modify the DNA of the repair deficient strain of *E. coli* p3478 ($polA^-$) when compared to DNA repair competent strain *E. coli* W3110 ($polA^+$)

Significant Toxicity Observed - Flunixin meglumine produced a significant difference between the zones of inhibition of the DNA repair deficient strain p3478, compared to the DNA repair competent strain W3110 in the absence and presence of S-9 mix. In the absence of S-9, the inhibition was dose dependent from doses of 2500 to 10,000 µg/plate. In the presence of S-9, the inhibition was limited to the high dose of 10,000 µg/plate.

No Observed Effect Level - not applicable

Statistical Analysis - not applicable

Conclusions - The results of this test indicate that flunixin meglumine can preferentially alter cellular DNA and cause primary DNA damage in the test organism. The positive activity seen in both DNA polymerase tests was reproducible and was obtained in independent laboratories.

7. Genotoxicity Study - Mouse Lymphoma - Flunixin Meglumine

Report Number A-18271

Study Date - February 14, 1984 to May 16, 1984

Study Director - Donald G. Campbell

Location of Study -Inveresk Research International
Musselburgh, Scotland

Identification of Substance and Dosage Form - Flunixin meglumine powder

Species and Strain - Mouse Lymphoma cell line, L5178Y TK+/- 3,7,2,C

Number of Animals Per Sex Per Treatment Group - not applicable

Drug Levels Tested and Duration of Dosing - First Experiment: 0, 31, 63, 125, 250, 500 and 1000 µg/mL in the absence of S-9 mix; Second Experiment: 0,

23, 45, 90, 180, 360 and 720 µg/mL in the presence of S-9 mix; Third Experiment: 0, 200, 250, 300, 350, 400, 450 and 500 µg/mL in the presence of S-9 mix.

Route of Drug Administration - not applicable

Parameters Tested - Tested for mutagenic activity in the mouse lymphoma L5178Y assay, both with and without a rat liver preparation and the co-factors necessary for mixed-function oxygenase activity (S-9 mix).

Significant Toxicity Observed - Mutagenic responses significantly higher than solvent control levels were found in repeated assays conducted in independent laboratories. In the first laboratory, dose-related increases in mutation frequency (up to 2-fold over solvent controls) were observed both with and without S-9 mix. In the second laboratory, 3- to 6-fold increases in mutation frequency were observed both with and without S-9 mix. Dose levels in excess of 500 µg/mL were too toxic for evaluation of mutagenic potential, while acceptable levels of toxicity occurred at lower doses which also caused the observed elevations in mutagenic activity.

No Observed Effect Level - not applicable

Statistical Analysis - not applicable

Conclusions - Flunixin meglumine was reproducibly mutagenic in mouse lymphoma L5178Y cells, both in the absence and presence of S-9 metabolism, as determined in independent laboratories.

8. Genotoxicity Study - Unscheduled DNA Synthesis - Flunixin Meglumine

Report Number A-18272

Study Date - March 9, 1984 to May 16, 1984

Study Director - D. B. McGregor, Ph.D.
Inveresk Research International
Musselburgh, Scotland

Identification of Substance and Dosage Form - Flunixin meglumine powder

Species and Strain - rat hepatocytes (adult male Fisher 344 rats)

Number of Animals Per Sex Per Treatment Group - not applicable

Drug Levels Tested and Duration of Dosing - 8, 16, 31, 125, 250, 500 and 1000 µg/mL plus controls (positive and negative)

Route of Drug Administration - not applicable

Parameters Tested - assesses the potential of a compound to induce unscheduled DNA synthesis. Silver grain counts in photographic emulsion formed by radiation from [6^{3}H]-thymidine taken up by a young, adult, male rat hepatocyte cells were examined.

Significant Toxicity Observed - The BANAMINE® dose groups did not meet any of the evaluation criteria for unscheduled DNA synthesis.

No Observed Effect Level - not applicable

Statistical Analysis - not applicable

Conclusions - BANAMINE® did not induce unscheduled DNA synthesis in adult rat hepatocyte primary cultures when tested to a concentration of 1000 µg/mL.

9. Genotoxicity Study - Chromosomal Aberrations - Flunixin Meglumine

Report Number A-18463

Study Date - May 1, 1984 to July 3, 1984

Study Director - D. B. McGregor, Ph.D.
Inveresk Research International
Musselburgh, Scotland

Identification of Substance and Dosage Form - Flunixin meglumine powder

Species and Strain - Chinese Hamster Ovary cells (CHO)

Number of Animals Per Sex Per Treatment Group - not applicable

Drug Levels Tested and Duration of Dosing - 25, 50, 100, 200, and 400 µg/mL plus controls (positive and negative) for either 6 hours in the presence of S-9 mix (mixed function oxygenase activity) or 24 hours in the absence of S-9 mix. A 6-hour exposure to 100, 200, and 400 µg/mL in the presence of S-9 mix was also examined in an independent test.

Route of Drug Administration - not applicable

Parameters Tested - to determine chromosomal aberrations and mutations. All structural chromosomal aberrations (lesions) were examined.

Significant Toxicity Observed - Significant positive clastogenic responses were recorded at 200 and 400 μg/mL (2- to 4-fold and dose related) in the presence of S-9 mix, and at 50, 100, and 200 μg/mL (2- to 5-fold) in the absence of S-9 mix. At 200 and 400 μg/mL in the absence of S-9 mix, flunixin meglumine was highly toxic to CHO cells. Increases (3- to 4-fold) in numerical chromosomal aberrations (endopolyploidy) were also observed at 200 and 400 μg/mL in the presence of S-9 mix, and excessive cytotoxicity was not observed.

No Observed Effect Level - not applicable

Statistical Analysis - not applicable

Conclusions - Flunixin meglumine was capable of reproducibly inducing chromosomal damage to CHO cells in vitro both in the absence and presence of S-9 metabolism.

10. Carcinogenicity Study in Rats

Report Number - P-4787

Study Dates - October 11, 1976 to October 13, 1978

Study Director- Bernard F. Murphy
Schering-Plough, Lafayette, NJ

Identification of Substance and Dosage Form - flunixin meglumine powder

Species and Strain - Charles River Sprague-Dawley rats

Number of Animals Per Sex Per Treatment Group - 60, the control group consisted of 100 animals per sex.

Drug Levels Tested and Duration of Dosing - 0, 0.6, 2.0, and 6.0 mg flunixin•NMG/kg/day (equivalent to 0, 0.36, 1.2, 3.6 mg flunixin /kg/day) was administered as a dietary admixture for up to 104 weeks. (For the first 4 weeks the rats were dosed at 1, 2, and 4 mg flunixin•NMG/kg/day intramuscularly.)

Route of Drug Administration - oral (diet)

Parameters Tested - clinical signs, body weight, food consumption, gross and microscopic pathology.

Significant Toxicity Observed -increased mortality at 3.6 mg flunixin/kg, and increased incidence of gastrointestinal lesions were noted in all treated groups.

Conclusions - Administration of flunixin meglumine to Sprague-Dawley rats for approximately 104 weeks had no carcinogenic effects. Because there was no serial hematology sampling in this study, a 1-year oral study in rats was requested so that potential hematopoietic toxicity could more fully be evaluated. The results of the 1-year study were previously described in this document.

11. Carcinogenicity Study in Mice

Report Number - P-5403

Study Dates - August 13, 1987 to June 27, 1990

Study Director- Darcy G. Perkins
Schering-Plough, Lafayette, NJ

Identification of Substance and Dosage Form - flunixin meglumine powder

Species and Strain - CD-1 mice

Number of Animals Per Sex Per Treatment Group - 60

Drug Levels Tested and Duration of Dosing - 0, 0.6, 2.0, and 6.0 mg flunixin meglumine/kg/day (equal to 0, 0.36, 1.2, and 3.6 mg flunixin free acid/kg/day), for 97 weeks.

Route of Drug Administration - oral via dietary admixture.

Parameters Tested - clinical signs, body weight, food consumption, hematology, gross and microscopic pathology were evaluated.

Significant Toxicity Observed -increased mortality and incidence of gastrointestinal lesions at 1.2 and 3.6 mg flunixin/kg. Transient decreased body weight gain at 3.6 mg flunixin/kg.

Conclusions - Administration of flunixin meglumine to CD-1 mice for approximately 97 weeks had no carcinogenic effects.

B. Calculation of an ADI and Safe Concentration for Flunixin

The initial toxicology and residue chemistry data submitted on flunixin permitted the establishment of a negligible tolerance of 0.1 ppm for each edible tissue. Current human food safety assessment procedures establish a finite tolerance for drug

residues based upon toxicology studies. While some of the toxicology studies conducted for flunixin do not meet current standards, the information is sufficient to establish an acceptable daily intake (ADI) and safe concentration. The most appropriate study for establishing the ADI is the two-year carcinogenicity study in rats. The LOEL in this study was 0.36 mg flunixin free acid/kg/day based on significant toxicity including gastrointestinal lesions which are typical of this class of compounds.

Using a LOEL of 0.36 mg/kg/day and a safety factor of 500, an ADI of 0.72 µg/kg bw/day or 43 µg/person/day (based on a 60 kg person) is calculated. The 500-fold safety factor rather than the traditional 100-fold safety factor for a chronic study was applied because treatment-related effects were seen at the lowest administered dose. Approximately 30% of the ADI, i.e. 13 ug/person/day, is reserved for milk. The remaining 70% of the ADI, i.e., 30 µg/person/day is applied to each edible tissue to calculate the safe concentration. Assigned values are summarized in Table 5.1.

Table 5.1. ADI, Consumption Factor, and Safe Concentration for flunixin free acid.

Tissue	ADI (µg/person/day)	Consumption Factor	Safe Concentration (flunixin free acid)
Muscle	30	300 g	0.1 ppm
Liver	30	100 g	0.3 ppm
Kidney	30	50 g	0.6 ppm
Fat	30	50 g	0.6 ppm

C. Total Residue Depletion and Metabolism Studies

1. SCH 14714•NMG: Metabolism Study of ^{14}C-Flunixin in Cattle. Study No. 93704

   a. Name and Address of Investigator:

      Test Facility (In-Life): John Byrd, Ph.D.

Principal Investigator
Southwest Bio-Labs, Inc.
401 N. 17th St., Suite 11
Las Cruces, NM 88005

Analytical Laboratory: S. Nilgun Comezoglu, Ph.D.
Study Director
XenoBiotic Laboratories, Inc.
107 Morgan Lane
Plainsboro, NJ 08536

b. Test Animals: Three crossbred cattle, 5 months old and weighing 430-470 lbs: 2 (M,F) test, 1 (M) control.

c. Route of Drug Administration and Time/Duration of Dosing:

Test animals were dosed intravenously via the jugular vein for three consecutive days with 3.53 mg ^{14}C-flunixin •NMG/kg/day. The test animals were terminated 18 hr post-final dose.

d. Radioisotope: ^{14}C-Flunixin•NMG (flunixin, N-methyl glucamine salt) was labeled in the trifluoromethyl carbon. Radiochemical purity: 98.06% (HPLC). Specific activity: 11.89 μCi/mg flunixin NMG.

e. Metabolism of ^{14}C-Flunixin•NMG in cattle: Flunixin was readily metabolized and eliminated: ca. 71% of the administered dose was recovered in urine (36%) and feces (35%), while liver and kidney tissues contained ca. 0.5% and 0.1% of the total radioactivity dosed, respectively.

Organo-extractable radioactivity accounted for 82-92% of the total radioactivity in liver and kidney tissues at 18 hr post-final dose. The major ^{14}C-residues (as % of the total radioactive residue, and ppm) in liver and kidney tissues (male and female) included flunixin (56-68%, 1.2-3.5 ppm), 5-hydroxy flunixin (0.5-1.6%, 0.011-0.085 ppm), 4'-hydroxy flunixin (0.42-1.4%, 0.013-0.071 ppm), and flunixin methyl ester (3.8-5.8%, 0.10-0.30 ppm). Details of metabolite distribution in liver and kidney tissues as determined by two-dimensional thin layer chromatography are shown in Table 5.2. Urine and feces were found to contain flunixin as a major component (54-69%), with the exception of female urine which contained flunixin as an acid-labile carboxyl-conjugate (78%).

Table 5.2
Percent Distribution of ^{14}C-Flunixin-NMG and Metabolites in Cattle Following Administration of ^{14}C-Flunixin-NMG

					Total Residue (ppm)			
					Liver		**Kidney**	
					M	**F**	**M**	**F**
					3.26	1.36	1.88	1.38
	% of Total Radioactive Residue (TRR)							
	Urine		**Feces**		**Liver**		**Kidney**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Metabolite								
flunixin (fln)	53.67	18.23	69.08	67.74	65.5	56.8	68.3	57.8
4'-OH fln	ND[b]	ND	ND[b]	ND[b]	1.32	1.37	0.42	ND
5-OH fln	3.65	3.39	ND	ND	1.82	0.64	0.93	0.48
2'-MeOH fln	ND[b]	ND[b]	ND	ND	ND	ND	ND	ND
fln conjugates [a]	42.68	78.38	22.09	20.84	8.74	10.83	12.48	17.76
fln methyl ester (unknown 1)	ND	ND	ND	ND	5.6	4.5	3.8	5.8
unknown 2	ND	ND	ND	ND	0.44	ND	ND	ND
unknown 4	ND	ND	ND	ND	ND	0.24	ND	ND
unknown 6	ND	ND	ND	ND	ND	ND	0.50	ND

a: Contains conjugates of flunixin, 4'-hydroxy flunixin and/or 5'-hydroxy flunixin (unknowns #3 and #5).

b: Metabolite was not detected by TLC analysis, however reconstructed HPLC radiochromatograms contained a radioactive peak with a retention time similar to the metabolite reference standard.

ND = not detected
M = Male
F = Female

2. SCH 14714: A Total Residue Depletion Study in Cattle Following Intravenous Administration of [^{14}C]-SCH 14714. Study No. 95708

   a. Name and Address of Investigator:

   Test Facility (In-Life): Charles E. Heird, Ph.D.
   Study Director
   Southwest Bio-Labs, Inc.
   401 N. 17th St., Suite 11
   Las Cruces, NM 88005

   Analytical Laboratory: Matthew J. Wisocky, B.S.
   Principal Investigator
   XenoBiotic Laboratories, Inc.
   107 Morgan Lane
   Plainsboro, NJ 08536

   b. Test Animals: 13 Hereford crossbred cattle, 5-9 months old and weighing 181-280 lbs were distributed into four groups of three animals each plus a control.

   c. Route of Drug Administration and Time/Duration of Dosing:
   Test animals were dosed intravenously via the jugular vein for three consecutive days with 3.6 mg ^{14}C-flunixin/kg/day. The animals were sacrificed by group at 24, 48, 72 and 96 hr post-final dose.

   d. Radioisotope: ^{14}C-Flunixin labeled in the trifluoromethyl carbon was used in this study. The radiochemical purity of the test article was 98.95% as determined by high-performance liquid chromatography. Specific activity: 0.579 µCi/mg.

   e. Total Residue Concentration: Urine and feces were collected throughout the study. At necropsy, the following edible tissue samples were collected: liver, kidneys, muscle (composite of leg and loin) and peritoneal fat. Combustion and quantitation of ^{14}C-content by liquid scintillation analysis afforded the results shown in Table 5.3. Residue values are expressed as ppm flunixin free acid.

Table 5.3. [^{14}C]-SCH 14714 Equivalent Residue Concentrations in Tissues (ppm ± standard deviation).

Time Post Final Dose (hr)	Liver (ppm)	Kidney (ppm)	Fat (ppm)	Muscle (ppm)
24	1.95 ± 0.66	1.42 ± 0.59	0.076[a]	0.023± 0.002
48	0.52 ± 0.07	0.47 ± 0.10	0.053[a]	NQ[b]
72	0.44 ± 0.07	0.37 ± 0.07	NQ	NQ
96	0.39 ± 0.09	0.25 ± 0.08	NQ	NQ

[a] One of three tissues was quantifiable.
[b] NQ = Not Quantifiable: Limits of detection were calculated based on a 2 Sigma, 95% confidence level in LSC analyses. Minimal detectable ppm were found to be 0.021 ppm (liver), 0.021 ppm (kidney), 0.039 ppm (fat) and 0.019 ppm (muscle).

Recoveries of ^{14}C-radioactivity in urine and feces for the four dose groups averaged 37-40% and 36-46%, respectively.

f. Methanol Extractability of Liver and Kidney ^{14}C-Residues:

The methanol extractability of total ^{14}C-residues in liver and kidney tissues as determined and is summarized in Table 5.4.

g. Flunixin Concentrations in Liver: The validated determinative HPLC assay for the analysis of flunixin (free acid) in liver tissues was conducted on the liver tissues, and the results are compared with total ^{14}C-residues in Table 5.5.

Table 5.4. Methanol Extracted and Bound [^{14}C]-SCH 14714 Equivalent Residues In Liver and Kidney Tissues (Percentages are given as means corrected for overall recovery).

LIVER		
Time Post Final Dose (hr)	**% Extracted (Methanol)**	**% Bound (Residue)**
24	67.3	32.7
48	32.1	67.9
72	31.4	68.6
96	23.3	76.7
KIDNEY		
Time Post Final Dose (hr)	**% Extracted (Methanol)**	**% Bound (Residue)**
24	65.3	34.7
48	47.7	52.3
72	42.7	57.3
96	50.3	49.7

Table 5.5. Total Radioactive Residues in Liver as Determined by Combustion and Flunixin Concentrations as Established by the Determinative Assay (Mean +/- standard deviation)

Time Post Final Dose (hr)	Total Residue (ppm)	Determinative Method (ppm)
24	1.95 +/- 0.66	0.844 +/- 0.51
48	0.52 +/- 0.067	0.146 +/- 0.029
72	0.44 +/- 0.068	0.111 +/- 0.049
96	0.39 +/- 0.085	0.109 +/- 0.028

D. Comparative Metabolism in the Rat

1. SCH 14714•NMG: Distribution, Metabolism and Excretion of ^{14}C-SCH 14714•NMG in Rats Following Oral Administration for Seven Consecutive Days. Study No. 94702

   a. Name and Address of Investigator:

      Diana Wu, Ph.D.
      Study Director
      XenoBiotic Laboratories, Inc.
      107 Morgan Lane
      Plainsboro, NJ 08536

   b. Test Animals: Six rats (3M, 3F, Sprague-Dawley, Crl:CD:BR, 6-10 weeks old, weighing 175-250 g); 2 additional rats (M,F) served as controls.

   c. Route of Drug Administration and Time/Duration of Dosing:

      Each test animal received ca. 10 mg ^{14}C-Flunixin•NMG/kg by gavage each day for seven days. The rats were sacrificed 2 hr post-final dose.

   d. Study Design: Urine and fecal samples collected during the in-life portion of the study were pooled by sex and analyzed by HPLC or TLC. The rats were sacrificed 2 hours post final dose and the radioactivity determined in the excreta and liver and kidneys (pooled by sex). Liver and kidneys were analyzed by TLC.

   e. Results: Flunixin was readily metabolized and eliminated from the rat. Total cumulative ^{14}C-dose eliminated in the urine and feces was 33-40% and 39-40%, respectively (similar for both males and females). The ^{14}C-residues identified in the excreta and tissues included free and conjugated forms of flunixin, 4'-hydroxy flunixin and 5-hydroxy flunixin, as well as 2'-methylhydroxy flunixin and flunixin methyl ester. Metabolic profiles were similar in both male and female rats. Characterized metabolites are listed in Table E as % total radioactive residue (%TRR) in urine, feces, liver and kidney.

      A comparison of the cattle liver and kidney flunixin metabolite data in Table 5.2 with the rat metabolism data in Table 5.6 showed that three very minor metabolites (Unknowns 2, 4 and 6) were observed in cattle tissues but were not seen in any of the chromatograms of rat tissues or excreta. Because of the small amounts of the metabolites present and the good match of the major metabolites of flunixin between cattle and rats, Unknowns 2, 4, and 6 were judged not to be of human safety concern. Thus, the rats used in the

toxicity evaluation of flunixin were exposed to all of the major metabolites appearing in cattle liver and kidney.

Table 5.6

Percent Distribution of ^{14}C-Flunixin-NMG and Metabolites in Rats Following Administration of ^{14}C-Flunixin-NMG

% of Total Radioactive Residue (TRR)								
Rats								
Metabolite	**Urine**		**Feces**		**Liver**		**Kidney**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
flunixin (fln)	57.1	50.0	15.0	14.2	87.1	82.3	91.0	69.0
4'-OH fln	1.9	1.7	6.6	8.1	ND	ND	ND	ND
5-OH fln	1.2	7.8	4.7	3.6	0.01	1.7	1.7	ND
2'-MeOH fln	10.2	10.1	11.6	7.8	ND	0.59	0.38	ND
fln conjugates [a]	15.2	15.6	19.4	26.4	ND[b]	ND	ND	ND
fln methyl ester (unknown 1)	ND	ND	ND	ND	0.38	0.05	0.46	11.3
unknown 2	ND	ND	ND	ND	ND	ND	ND	ND
unknown 4	ND	ND	ND	ND	ND	ND	ND	ND
unknown 6	ND	ND	ND	ND	ND	ND	ND	ND

a: Contains conjugates of flunixin, 4'-hydroxy flunixin and/or 5'-hydroxy flunixin (unknowns #3 and #5).
b: Metabolite was not detected by TLC analysis, however reconstructed HPLC radiochromatograms contained a radioactive peak with a retention time similar to the metabolite reference standard.
ND = not detected
M = Male
F = Female

E. Selection of Target Tissue and Marker Residue

The total residue measurements in studies SN 93704 and SN 95708 with ^{14}C-flunixin in cattle established that liver is the edible tissue of cattle in which residues of flunixin are highest and persist the longest relative to its safe concentration. The metabolism data in those two studies showed that parent flunixin is the most abundant drug related residue in liver tissue and that strong acid hydrolysis enhances recovery by release of bound and/or conjugated residues. Those observations led to the development and validation of a regulatory assay for flunixin based on parent flunixin as the marker residue and liver as the target tissue.

F. Bioavailability of Bound ^{14}C-Residues in Cattle Liver

The bioavailabilities of parent flunixin and incurred bound residues of flunixin in cattle liver were determined in the rat in an attempt to discount from human food safety concern a portion of the cattle liver total residue.

1. SCH 14714 (Flunixin): Bioavailability of ^{14}C-SCH 14714 Residues in Rats Receiving Liver from ^{14}C-SCH 14714•NMG-Dosed Cattle. Study No. 92702

   a. Name and Address of Investigator:

   Test Facility (Cattle In-Life): John Byrd, Ph.D.
   Principal Investigator
   Southwest Bio-Labs, Inc.
   401 N. 17th St., Suite 11
   Las Cruces, NM 88005

   Analytical Laboratory
   and Rat In-Life: Shawn F. Charles, M.S.
   Study Director
   Schering-Plough Research Institute
   144 Rte. 94 S.
   PO Box 32
   Lafayette, NJ 07848

   b. Test Animals:

   Cattle: Three crossbred cattle, 5 months old and weighing 430-470 lbs, 2 (M,F) test, 1 (M) control.

   Rats: Bile duct cannulated (Hla:(SD)CVF) supplied by Hilltop Lab Animals, Inc. with a closed-loop cannula surgically inserted at both ends of the common bile duct. Eight rats, 2M/2F comparative controls; 2M/2F test group.

   c. Route of Drug Administration and Time/Duration of Dosing:

   Cattle: Test animals were dosed intravenously via the jugular vein for three consecutive days with 3.53 mg ^{14}C-flunixin •NMG/kg/day. The test animals were terminated 18 hr post-final dose.

   Rats were fed pelletized, methanol-extracted cattle liver tissue either fortified with ^{14}C-Flunixin•NMG (comparative controls) or derived from cattle previously dosed with ^{14}C-Flunixin•NMG (test group). Urine, feces and bile were collected until sacrifice at 48 hr.

d. Radioisotope: ^{14}C-Flunixin•NMG was labeled in the trifluoromethyl carbon, >98% radiochemically pure; specific activity used in fortifications: 12.5 μCi/mg.

e. Bioavailability: The method used to determine bioavailability was based on that reported by Gallo-Torres (Journal of Toxicology and Environmental Health, 2:827-845, 1977). The ^{14}C-content of the following components was used to establish the relative bioavailability of bound ^{14}C-flunixin residues: urine, feces, bile, GI tract wall, GI tract contents, liver, kidneys, and carcass; the results are summarized in Table 5.7.

Table 5.7. Summary of Bioavailability Determinations (Results Expressed as % of Total ^{14}C-Dosed, Normalized to 100%)

	Matrix	Comparative Control (2M, 2F) (%)	Test Group (2M, 2F) (%)
Absorbed	Urine	18.4	9.2
	Bile	63.6	25.5
	GI-Tract Wall	0.5	1.6
	Liver	0.3	2.1
	Kidneys	0.03	0.8
	Carcass	1.1	0.0
	Total	84.0	39.2
Non-Absorbed	Feces	11.2	50.8
	GI Contents	0.1	2.2
	Total	11.3	53.1
% Relative Bioavailability		(39.2/84.0) x 100% =46.7%	

Relative bioavailability of bound ^{14}C-flunixin residues, defined as the percent ratio of absorbed radioactivity in the test group to that in the comparative control group was found to be 46.7%.

G. Assignment of the Target Tissue (Liver) Tolerance

A tolerance of 0.125 ppm was calculated for parent flunixin free acid (the marker residue) in cattle liver (the target tissue). That determination was made using the total residue and marker residue depletion data in study #95708 after a discount of a portion of the liver bound residue from human food safety concern. The discount of the liver bound residue was made using the bioavailability data in Section F (Bioavailability of Bound ^{14}C Residues in Cattle), and the general approach described in Gallo-Torres (Journal of Toxicology and Environmental Health, 2:827-845, 1977).

The bioavailability data (Section F) showed that the flunixin bound residue was less absorbed than parent flunixin with a relative bioavailability of 46.7%. The procedure to discount the liver bound residue involved calculation of values to define a total residue of concern (TRC) decline curve. The total residue of concern was calculated as follows.

TRC= Total Residue(ppm) x extractable fraction +
Total Residue(ppm) x unextractable fraction x relative bioavailability
where relative bioavailability = 46.7% (Study # 92702)

The results of those calculations are listed in Table 5.8.

Table 5.8.
Total Residues of Concern in Liver
(results expressed as the mean; three animals per time point).

	Liver Residues		
Withdrawal Time (hr)	Total (ppm)	Bound (%)	TRC (ppm± std dev)
24	1.95	32.7	1.63 ±0.64
48	0.52	67.9	0.33 ±0.03
72	0.44	68.6	0.28 ±0.05
96	0.39	76.7	0.23 ±0.05

The relationship between the total residues of concern in liver and free flunixin, as quantitated by the validated assay, is shown in Table 5.9 using data generated in the total residue depletion study (Study No. 95708).

Table 5.9. Total Residue Depletion Results: Total Residues of Concern and Free Flunixin (Determinative Assay) in Liver

Withdrawal Time (hr)	Total Residues of Concern (TRC) (ppm)	Free Flunixin (Assay Results) (ppm)	Ratio: Flunixin/TRC (±std dev)
24	1.63	0.844	0.481 ±0.145
48	0.33	0.146	0.453 ±0.125
72	0.28	0.111	0.380 ±0.104
96	0.23	0.109	0.473 ±0.034

To obtain the tolerance, the TRC curve was plotted along with the marker residue values in liver tissue from Study #95708. The tolerance (0.125 ppm) was obtained as the value on the marker residue decline curve at the point in time when the TRC curve was at the safe concentration for flunixin total residues in cattle liver (0.3 ppm).

H. Assignment of the Tolerance for Residues of Flunixin in Muscle

The total residue studies conducted for this NADA have shown that, under the approved conditions of use for flunixin·NMG in cattle, peak levels of flunixin total residues in muscle tissue are in the range of 0.020 to 0.025 ppm in the first one or two days post dosing. These peak values are well below the safe concentration of 0.1 ppm in muscle.

Although it was not directly measured in cattle muscle in the residue studies, parent flunixin free acid in muscle is expected to be in the range of 0.004 to 0.008 ppm, or about 25 to 40% of the total residue, based on metabolite profiling of flunixin residues in liver and kidney. At the regulated withdrawal time of four days, parent flunixin is undetectable in cattle muscle tissue by the currently available analytical methods for the drug. To ensure the safety of the food supply, a tolerance of 0.025 ppm is established for residues of parent flunixin free acid in cattle muscle.

I. Study to Establish the Withdrawal Time

1. SCH 14714: A Final Residue Depletion Study in Cattle Following Intravenous Administration of Flunixin•NMG. Study No. 96219

   a. Name and Address of Investigator:

   Test Facility (In-Life): Charles E. Heird, Ph.D.
   Study Director
   Southwest Bio-Labs, Inc.
   401 N. 17th St., Suite 11
   Las Cruces, NM 88005

   Analytical Laboratory: Robert Robinson, Ph.D.
   XenoBiotic Laboratories, Inc.
   107 Morgan Lane
   Plainsboro, NJ 08536

b. Test Animals: 26 crossbred beef cattle (13 M, 13F) were assigned to five test groups (5 cattle each) and 1 control (M).

c. Route of Drug Administration and Time/Duration of Dosing:

   Test animals were dosed intravenously via the jugular vein for three consecutive days with 3.6 mg Flunixin•NMG/kg/day. The animals were sacrificed by group at 48, 72, 96, 120 and 144 hr post-final dose.

d. Test Article: Flunixin NMG (flunixin meglumine, BANAMINE®) at a concentration of 50 mg (flunixin free acid)/mL as the commercial, market-ready formulation.

e. Liver Residue Concentrations: Concentrations of free flunixin in liver tissues were determined using a validated assay (Study No. 95703) and were found to decrease from a mean of 0.094 ppm at 48 hr to 0.042 ppm at 144 hr post-final dose (Table 5.10).

Table 5.10. Summary of Determinative Analyses for Flunixin in Bovine Liver Tissues Obtained from Cattle Administered 2.2 mg Flunixin /kg/day Intravenously for Three Days

Group	Withdrawal Time (hr)	Flunixin Concentration (ppm) Mean ± SD
I	48	0.094 ± 0.0166
II	72	0.096 ± 0.0118
III	96	0.061 ± 0.0084
IV	120	0.048 ± 0.0085
V	144	0.042 ± 0.0096

Calculation of Withdrawal Time

Using the target tissue (liver) tolerance of 0.125 ppm and the flunixin liver residue data in Table I, a withdrawal time of 4 days was calculated for this use of flunixin meglumine in cattle. The withdrawal time was calculated using the Agency's statistical tolerance limit method (99% tolerance limit with a 95% confidence interval method).

J. Regulatory Methods

1. Determinative Assay Procedure

The determinative assay for the marker residue, flunixin, is a high-performance liquid chromatography (HPLC) method which provides acceptable sensitivity, specificity, accuracy and precision for the routine monitoring of flunixin residues in bovine liver. Flunixin, present in liver tissue as free, conjugated or esterified forms, is hydrolytically released, extracted, and purified through the sequential use of silica gel, reversed-phase, and cation exchange column chromatography. The purified solution is analyzed by HPLC using a mobile phase containing an ion pairing reagent. The method was demonstrated to reliably quantitate flunixin residues at levels of 0.01-0.20 ppm. No interference was observed from 17 veterinary drugs commonly used in cattle.

2. Confirmatory Assay Procedure

The confirmatory method utilizes a liquid chromatography/mass spectrum/mass spectrum (LC/MS/MS) methodology applied to the purified solution obtained from the determinative method work-up. Daughter ion (m/z 297) mass spectrometry yielded confirmatory ions at m/z 264, 279 (base peak), and 297.

The validated regulatory method for detection of residues of flunixin is filed in the Food additives Analytical Manual on display in FDA's Freedom of Information Public Room (Room 12A-30), 5600 Fishers Lane, Rockville, MD 20857.

VII. AGENCY CONCLUSIONS

The data submitted in support of this supplemental NADA comply with the requirements of section 512 of the Act and demonstrate that flunixin meglumine injection, when used under the proposed conditions of use, is safe and effective for the control of pyrexia associated with bovine respiratory disease and endotoxemia and for the control of inflammation in endotoxemia in beef and nonlactating dairy cattle when administered intravenously at 1.1 to 2.2 mg/kg daily for up to 3 days.

Based on a battery of toxicology tests, the safe concentrations for total residue of flunixin free acid are: 0.1 ppm in muscle, 0.3 ppm in liver, 0.6 ppm in kidney, and 0.6 ppm in fat. The metabolism studies in cattle conducted for this NADA provided data that was used to assign a tolerance of 0.125 ppm for the marker residue, flunixin free acid, in the target tissue, cattle liver. The tolerance refers to the residue measured by the regulatory method described herein. A tolerance of 0.025 ppm is also established for residues of flunixin free acid in cattle muscle.

A pre-slaughter withdrawal period of 4 days was calculated from a residue depletion study of flunixin residues in cattle, following the administration of BANAMINE® Injectable Solution.

Labeling restricts this drug to use or on the order of a licensed veterinarian. This decision was based on the following factors: (a) the product contains a new nonsteroidal antiinflammatory agent intended for therapeutic purposes and (b) adequate directions cannot be written to enable lay persons to appropriately decide in which animals this drug may be used safely.

In accordance with 21 CFR 514.106(b)(2)(vii), this is a category II change. The approval of this change did not require a reevaluation of the safety and effectiveness data in the parent application.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment, which may be seen in the Docket Management Branch (HFV-305), Park Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20855.

Under section 512(c)(2)(F)(iii) of the FFDCA, this approval for food producing animals qualifies for THREE years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the application and conducted or sponsored by the

applicant. The THREE years of marketing exclusivity applies only to the new indication for the control of pyrexia associated with bovine respiratory disease and endotoxemia and for the control of inflammation in endotoxemia in beef and non-lactating dairy cattle. There are currently no patents in effect which pertain to flunixin meglumine or BANAMINE® Injectable Solution.

VIII. Approved Product Labeling

Facsimile package insert and labeling for the 50 mL, 100 mL, and 250 mL vials and box carton.