DATE OF APPROVAL: AUGUST 19, 2004

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 101-479

BANAMINE Injectable Solution
(Flunixin meglumine)

This supplement allows for use in lactating dairy cattle for the existing indications of "the control of pyrexia associated with bovine respiratory disease and endotoxemia and for the control of inflammation in endotoxemia." Additionally, it allows for a new indication "for the control of pyrexia associated with acute bovine mastitis" and it establishes a tolerance for residues of flunixin in milk.

Sponsored by:
Schering-Plough Animal Health Corp.

1. GENERAL INFORMATION:

a. File Number:	NADA 101-479
b. Sponsor:	Schering-Plough Animal Health Corp. 1095 Morris Ave. Union, NJ 07083 Drug Labeler Code: 000061
c. Established name:	Flunixin meglumine
d. Proprietary name:	BANAMINE Injectable Solution
e. Dosage Form:	Injectable solution
f. How Supplied:	50, 100, and 250 mL multi-dose vials
g. How Dispensed:	Rx
h. Amount of Active Ingredients:	50 mg flunixin/mL
i. Route of Administration:	IV
j. Species/Class:	Cattle/Beef and non-lactating dairy cattle
k. Recommended Dosage:	1.1 to 2.2 mg/kg (0.5 to 1 mg/lb; 1 to 2 mL per 100 lbs) of body weight 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 (1.0 mg/lb) of body weight. Avoid rapid intravenous administration of the drug.
l. Pharmacological category:	Nonsteroidal anti-inflammatory drug (NSAID)
m. Indications:	Currently approved 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.
n. Effect of the Supplement:	To allow for use in lactating dairy cattle for the existing indications of "the control of pyrexia associated with bovine respiratory disease and

endotoxemia and for the control of inflammation in endotoxemia." To allow the addition of a new indication “for the control of pyrexia associated with acute bovine mastitis.” The recommended dosage for the new indication is 2.2 mg/kg (1 mg/lb; 2 mL per 100 lbs) of body weight given once by intravenous administration. To establish a tolerance for residues of flunixin in milk at 2 ppb.

2. EFFECTIVENESS:

a. Dosage Characterization:

Effectiveness studies were presented in the supplemental NADA 101-479 FOI Summary approval dated May 6, 1998, establishing the recommended effective dose of BANAMINE Injectable Solution for the control of pyrexia associated with bovine respiratory disease and endotoxemia and control of inflammation associated with endotoxemia in beef and non-lactating dairy cattle.

b. Substantial Evidence:

No further effectiveness data were required to allow for use in lactating dairy cattle for the indications previously approved in beef and non-lactating dairy cattle. The effectiveness of BANAMINE Injectable Solution for the control of pyrexia associated with acute bovine mastitis was demonstrated in the following multi-site clinical field trial.

Study # C00-158-00 – Title: The clinical efficacy of flunixin meglumine for the management of pyrexia and/or inflammation associated with naturally occurring acute bovine mastitis

1) Type of Study: Multi-site field trial in dairy cows with acute bovine mastitis

2) Investigators

Site 01: Dr. Joe Hogan, Ohio State University, Wooster, OH

Site 05: Dr. Phil Sears, Michigan State University, East Lansing, MI

Site 06: Dr. Kevin Anderson, North Carolina State University
Raleigh, NC

3) General Design

a. Objective: To evaluate the effectiveness of flunixin meglumine (BANAMINE Injectable Solution; SPAHC) for the management of pyrexia and/or inflammation associated with acute bovine mastitis. The dosage of flunixin was 2.2 mg/kg body weight, administered intravenously once (upon enrollment).

b. Animals: A total of one hundred and seventeen adult lactating dairy cows, mainly Holsteins, were enrolled in this study.

c. Treatment Groups: There were 2 treatment groups. The cows were randomly allocated to Control (saline) or Treatment (flunixin) groups. Fifty-eight (58) cows were enrolled in the flunixin treatment group. Fifty-nine (59) cows were

enrolled in the negative control (saline) group.

d. Inclusion Criteria: Cows were enrolled when diagnosed with acute mastitis as evidenced by having an elevated rectal temperature ≥ 104.0°F (≥ 40.0°C) and showing at least 2 of the clinical signs of udder inflammation: swelling, pain, or firmness. Cows with one or two affected quarters were considered as having the same disease severity.

e. Dosage Form: BANAMINE Injectable Solution containing 50 mg flunixin per mL as the meglumine salt.

f. Route of Administration: Intravenous injection (IV)

g. Dose: BANAMINE Injectable Solution at 1 mL/50 lb (2.2 mg/kg) or physiological saline at 1 mL/50 lb; given once, IV in the jugular vein.

h. Test Duration: 4 hours

i. Pertinent Parameters Measured: The primary variables for this study were treatment Success/Failure rates for pyrexia and inflammation. A cow was designated a treatment success for pyrexia when the rectal temperature decreased ≥2°F (1.1 °C) from the inclusion rectal temperature or the temperature of the cow had decreased to the normal rectal temperature indices as described in The Merck Veterinary Manual, [7th Edition, page 966] of 101.5 ± 1°F, 4 hours after administration of the test article. A cow was designated a treatment failure for pyrexia when the rectal temperature did not decrease ≥2°F (1.1°C) from the inclusion rectal temperature nor did the rectal temperature decrease to the normal indices as described in The Merck Veterinary Manual, [7th Edition, page 966] of 101.5 ± 1°F, 4 hours after administration of the test article.

Udder inflammation was assessed by clinical signs of pain, swelling, and firmness, which were assessed separately by visual observation and palpation. A four-tiered scale was used for each criteria with grade scores of 0 = normal, 1 = mild, 2 = moderate, and 3 = severe. A cow was designated a treatment success for inflammation when the selected quarter had 2 or more of the inflammatory parameters (pain, swelling, or firmness) improve by 2 or more grade scores or return to normal (grade score = 0), 4 hours after administration of the test article. A cow was designated a treatment failure, when the selected quarter did not have 2 or more of the inflammatory parameters (pain, swelling, or firmness) improve by 2 or more grade scores or return to normal, 4 hours after administration of the test article.

In addition, treatment success/failure for each individual inflammation variable (pain, swelling, and firmness) was analyzed using the same criteria as inflammation success/failure.

j. Statistical Analysis: The experimental unit was the individual cow (one or two affected quarters per cow enrolled). The analysis of treatment success/failure for pyrexia and inflammation were by a generalized linear mixed model with binomial errors and a default logit link.

4) Results: Eighteen (18) flunixin-treated cows were excluded from the evaluation of success/failure for pyrexia and inflammation due to non-compliance with the protocol. A total of 40 protocol-compliant cases in the treatment group were included in the analyses. Fourteen (14) control cows were excluded from the evaluation of success/failure for pyrexia and inflammation due to non-compliance with the protocol. A total of 45 protocol-compliant cases in the control group were included in the analyses.

Statistically significant higher success rates for the reduction of pyrexia were seen after test article treatment. The success rate in the BANAMINE treatment group was 95.0% as compared to 20.0% in the saline group ($p<0.0001$). There was no statistically significant difference in success rates for inflammation between the BANAMINE treatment group and the saline treatment group ($p = 0.0632$).

Key statistical assessments are summarized in **Table 2.1** below.

Table 2.1 Summary of Statistical Assessment

	BANAMINE	Saline	p value
Primary Variables			
Success rate for pyrexia	95.0%	20.0%	p < 0.0001*
Success rate for inflammation	8.1%	2.3%	p = 0.0632*
Secondary Variables			
Success rate for pain[§]	27.0%	4.5%	p = 0.0199*
Success rate for swelling[§]	13.5%	0.0%	p = 0.0170**
Success rate for firmness[§]	8.1%	4.3%	p = 0.1824*
Mean rectal temperature Time 0 hours	104.8°F	104.8°F	p = 0.8304***
Mean rectal temperature Time 4 hours	101.5°F	103.8°F	p = 0.0002****

[§]Components of the inflammation variable
*Generalized linear mixed model with binomial errors and a default logit link, one-sided p-value
** Wilcoxon Rank Sum Exact Test
*** mixed model, Analysis of Variance
**** mixed model, Analysis of Variance, one-sided p-value

5) Adverse Reactions: No drug related adverse events were observed during the

conduct of this study. One cow in the saline group died during the study, and one cow in the saline group was reported as being "toxic – cold ears, lethargic".

6) Conclusion: While the number of protocol compliant cases was lower than cows enrolled, it was deemed a sufficient number to provide substantial evidence for pyrexia associated with acute bovine mastitis for the following reasons:

- Effectiveness for the control of pyrexia has already been demonstrated in the supplemental approval dated May 6, 1998, when beef and non-lactating dairy cattle were originally added to the label;
- The number of animals available for analysis was well above the number of animals needed to obtain sufficient statistical power in this study;
- There was evidence of effectiveness as shown by the statistically significant difference between the BANAMINE treatment group and the control group in the pyrexia analysis ($p<0.0001$) using either per-protocol or intention-to-treat principles.

Under the conditions of this study, BANAMINE Injectable Solution is effective for the control of pyrexia associated with acute bovine mastitis.

3. TARGET ANIMAL SAFETY:

No further target animal safety data were required from the NADA 101-479 supplemental approval dated May 6, 1998.

4. HUMAN SAFETY:

A. Toxicity Tests

Toxicity studies with flunixin meglumine are described in the FOI summary made available when the NADA was codified on July 20, 1998 (63 FR 38749). These include mutagenicity, oral feeding, teratology, and carcinogenicity studies. The lowest no-observed-effect-level from these studies was 0.36 mg flunixin per kg body weight per day. The Acceptable Daily Intake (ADI) assigned on the basis of the toxicity studies was 0.72 micrograms per kg body weight per day.

B. Assignment of Safe Concentrations

The toxicity tests referenced above resulted in the following safe concentrations for total residues of flunixin (flunixin free acid) in tissues:

muscle:	0.1 ppm
kidney:	0.6 ppm
liver:	0.3 ppm
fat:	0.6 ppm

Approximately 30% of the ADI, i.e., 13 micrograms per person per day, is reserved for milk. The calculated safe concentration for total residues of flunixin in milk is 10 ppb (one tenth that of muscle).

C. Total Residue Depletion and Metabolism Study:

Summaries of all residue metabolism studies in tissues of beef and non-lactating cattle with flunixin meglumine are incorporated by reference to approved NADA No. 101-479 for the BANAMINE Injectable Solution.

For the current supplement to NADA 101-479, a total residue and metabolism study was conducted in lactating dairy cattle. That study is summarized below, and it reports the levels of parent flunixin and its metabolites that occur in milk following the treatment of lactating dairy cows with three once-daily doses of ^{14}C-flunixin·NMG.

Study Title:	"SCH 14714-NMG (Flunixin·NMG): A Milk Total Residue Depletion Study in Dairy Cattle Following IV Administration of ^{14}C-Flunixin Meglumine"
Study No:	98493
Study Director:	William F. Feely, M.S., Schering-Plough Research Institute, Lafayette, NJ
Investigators:	In-Life Testing Facility: John Campbell, Ph.D., Southwest Bio-Labs (SBL), Las Cruces, NM Analytical Facility: William F. Feely, M.S., Schering-Plough Research Institute, Lafayette, NJ
Animals:	Eight Holstein dairy cows (4 high-yielding in Group I and 4 low-yielding in Group II), 2-10 years old, and 490-610 kg at dosing.

Route of Drug Administration and Duration of Dosing

All dairy cattle (Groups I and II) were dosed by IV injection (jugular vein, 18 gauge catheter) once per day in the morning for three consecutive days (total of 3 doses) at a nominal level of 2.2 mg ^{14}C-flunixin free acid/kg per day. The actual dose level was 2.0 to 2.1 mg/kg per day.

Radioisotope

The test substance was obtained by diluting ^{14}C-flunixin·NMG with flunixin·NMG to a final specific activity of 8492 dpm/microgram flunixin free acid. The dosing solution was prepared by addition of the ^{14}C-labeled test substance (^{14}C-flunixin·NMG) to placebo (no flunixin·NMG) BANAMINE Injectable Solution. Radiochemical purity was greater than 99% by HPLC for the dosed flunixin. Radiochemical purity was greater than or equal to 99% by HPLC and TLC for the synthesized ^{14}C-flunixin. The final concentration of the dose solution was 48.9 mg flunixin (free acid)/mL.

Sample Collection

Whole milk was collected from each animal twice per day (AM and PM) beginning at the PM milking following the initial dose and continuing for nine days post-final dose in half the animals (Groups Ia and IIa) or 13 days post-initial dose for half the animals (Groups Ib and IIb). Urine and feces were collected pre-dose and at 24 hour intervals for nine days post-initial dose from one animal each from Groups I and II. All samples were stored at $\leq$ -17°C.

All animals were sacrificed by captive bolt pistol followed by exsanguination. At necropsy, the animals were grossly observed for the presence of any pathology. Following the examination, liver (whole), kidneys (both), muscle (loin), and fat (omental and subcutaneous) were taken. Approximately 500 g of each tissue were collected after the specimens were weighed and chopped.

Total Radioactive Residues in Milk

Residue levels of ^{14}C-flunixin in µg-equivalents/gram (ppm) found in milk following three IV injections of ^{14}C-flunixin·NMG were measured for all animals over a period of nine days. **Table 4.1** lists milk residue levels expressed as ppb ^{14}C-flunixin equivalents for examined milk. For the first three milkings after the last dose, residue levels ranged from 3 to 142 ppb. Except for animal #2903, total residue levels were less than 71 ppb for all animals at the first milking after administration of the last dose. By the fourth milking after administration of the last dose (Day 3, AM) total residue levels in all animals, except for animal #2903 (32 ppb), were less than or equal to 5 ppb. Therefore, residue levels in milk declined rapidly. Percentage of the total administered dose that was excreted in milk was less than 0.02%.

Table 4.1: Total ^{14}C-Flunixin Residues in Milk			
Animal #	**1st milking after last dose**	**2nd milking after last dose**	**3rd milking after last dose**
Group #	**(ppb)***	**(ppb)***	**(ppb)***
2901/I	56	10	5
2902/I	64	10	5
2905/I	71	9	5
2906/I	26	5	3
2896/II	63	13	7
2899/II	54	20	12
2900/II	53	9	4
2903/II	142	87	67
* ppb (μg/kg) ^{14}C-flunixin equivalents			

Measurement of Major Metabolites by HPLC Radiochromatography

Each milk sample, which contained sufficient radioactivity, was examined by HPLC radiochromatography for the presence of unchanged flunixin and its metabolites. For the first milking after the last dose, the major residue in milk was 5-hydroxy flunixin. The percent of 5-hydroxy flunixin present in the total radioactive residue ranged from 31 to 61% of HPLC radioactivity recovered, while amounts of parent flunixin ranged from 12 to 32%. Recovery of column radioactivity as 5-hydroxy flunixin was approximately 17 and 20% for the second and third milkings and for flunixin was 20 and 22% for the second and third milkings, respectively. Additional analyses also showed that a third flunixin residue, 4'-hydroxy flunixin co-eluted with small amounts of radioactivity.

Residue levels for 5-hydroxy flunixin measured by HPLC radiochromatography ranged from 77 to 0 (not detected) ppb for the milk samples analyzed. Residue levels for flunixin range from 27 to 0 (not detected) ppb.

Details of metabolite distribution in milk samples as determined by HPLC radio-chromatography are shown in **Table 4.2**. (Also see **Table 4.4**.)

Table 4.2: 5-Hydroxy Flunixin and Flunixin Residue Levels Measured by HPLC Radiochromatography					
Animal #/ Group #	**TRR (ppb)**	**5-OH flunixin**		**Flunixin**	
		%HPLC	ppb	%HPLC	ppb
1st milking after last dose					
2901/I	56	48	27	15	8
2902/I	64	61	39	15	10
2905/I	71	54	38	12	9
2906/I	26	46	12	14	4
2896/II	63	31	20	25	16
2899/II	54	34	18	32	17
2900/II	53	41	22	14	7
2903/II	142	54	77	15	21
2nd milking after last dose					
2901/I	10	18	2	14	1
2902/I	10	11	1	41	4
2905/I	9	25	2	14	1
2906/I	5	2	0	9	0
2896/II	13	12	2	18	2
2899/II	20	23	5	23	5
2900/II	9	12	1	10	1
2903/II	87	32	28	31	27
3rd milking after last dose					
2896/II	7	5	0	18	1
2899/II	12	23	3	18	2
2903/II	67	37	25	29	19

ppb (µg/kg) ^{14}C-flunixin equivalents
TRR - total radioactive residue
For the third milking, the animals not reported were below the limit of quantitation.

Analysis of Marker Residue in the Radiolabeled Milk Samples by the Determinative Procedure

The mean results of the analysis of the radiolabeled milk samples by the validated HPLC MS/MS determinative procedure for the marker residue, 5-hydroxy flunixin, from cattle treated with ^{14}C-flunixin meglumine are shown in **Table 4.3**. Concentrations of 5-hydroxy flunixin ranged from 35.7 ppb to below the limit of quantitation (BLQ) of 1 ppb for Day 0 samples, from 27.9 ppb to BLQ for Day 1 samples, and from 66.0 to 1.0 ppb for Day 2 samples. The majority of the

samples from Days 3 and 4 were BLQ. These results, as shown in the attached table, were compared to the milk total radioactive residues and the ratio of marker to total residue ranged from 0.11 to 0.66 for individual samples.

Table 4.3 Analysis of Radiolabeled Samples by the Determinative Method for the Marker Residue, 5-Hydroxy Flunixin in Bovine Milk

	Mean ppb Marker Residue [1]	Mean ppb total Residue [2]	Mean Ratio marker/ total [3]
Day 0 PM	**12.23**	**55.38**	**0.22**
1st milking after 1st dose			
Day 0 AM	**3.72**	**17.75**	**0.17**
2nd milking after 1st dose			
Day 1 PM	**18.31**	**65**	**0.30**
1st milking after 2nd dose			
Day 1 AM	**2.71**	**17.25**	**0.15**
2nd milking after 2nd dose			
Day 2 PM	**32.06**	**66.13**	**0.48**
1st milking after 3rd dose			
Day 2 AM	**4.43**	**20.38**	**0.20**
2nd milking after 3rd dose			
Day 3 PM	**9.05**[4]	**39.5**[4]	**0.23**
3rd milking after 3rd dose			
Day 3 AM	**5.5**	**32**	**0.17**
4th milking after 3rd dose			
Day 4 PM	**2**[5]	**17**	**0.12**
5th milking after 3rd dose			
Day 4 AM	**BLQ**	**9**[6]	**NA**
6th milking after 3rd dose			

BLQ – below limit of quantitation, 1 ppb
NA - not applicable
1 - from analyses of milk samples by determinative method
2 – from the radioactive analyses of the milk samples
3 – ppb marker residue/ppb total residue
4 – values were calculated from two animals; six animals were BLQ
5 – values were from one animal; seven animals were BLQ
6 – value from one animal

D. COMPARATIVE METABOLISM

Profiling of major and minor ^{14}C-flunixin metabolites present in milk was conducted in order to make a comparison of the profile in milk with the profile of ^{14}C-flunixin metabolites observed in the test species, the rat.

Metabolite Profiling in the Rat

A study of the metabolism and excretion of ^{14}C-flunixin meglumine in male and female Sprague-Dawley rats was conducted earlier for the approval of flunixin meglumine in beef cattle (NADA 101-479). In that work, each rat was administered *ca* 10 mg ^{14}C-flunixin meglumine/kg by gavage each day for seven consecutive days. The rats were sacrificed 2 hr post-final dose.

Samples collected during the in-life portion of the rat study were analyzed for total radioactivity and for flunixin metabolites present. For the latter, the samples were pooled by sex and were analyzed by reverse phase HPLC with a C18 column using a mobile phase of ammonium acetate in methanol/water. The HPLC chromatograph was equipped with UV and ^{14}C radioactivity detectors. Profiles of ^{14}C-flunixin metabolites in the rat were obtained for urine, feces, liver, and kidneys (all pooled by sex).

The results of the study in rats showed that flunixin was readily metabolized and eliminated by the rat. The total cumulative dose of ^{14}C-flunixin eliminated in the urine and feces was 33-40% and 39-40%, respectively and was similar for both males and females. The ^{14}C-flunixin metabolites identified or characterized in tissues and in excreta are listed in Table 4.4

Metabolite Profiling in Milk

Total residue measurements in milk as well as the levels of parent flunixin and 5-hydroxy flunixin reported in study 98493 are listed in **Section C**, **Total Residue and Metabolism Study**. Further radiochromatography was performed with the milk samples in order to profile all of the measurable ^{14}C-flunixin metabolites present in milk. The profiling was done with reverse phase HPLC with a C8 column using a mobile phase of ammonium acetate in methanol/water. One minute aliquots of the eluate from the column were collected, and the radioactivity present in each aliquot was measured by liquid scintillation counting. Metabolite identification was made by comparison of retention times with authentic samples and by co-chromatography with some of the known metabolites.

The milk samples profiled were from the first three milkings post-final dose with milking performed twice daily. The results of these analyses are shown in a side-by-side comparison in **Table 4.4** along with the results of the flunixin metabolite profiling in tissues, urine, and feces of the rat.

Comparison of the Flunixin Metabolites in Milk and in the Rat

The listing of metabolites in **Table 4.4** shows that flunixin and its three identified major metabolites in milk were also present in the tissues or excreta of rats, the test species. In addition to the identified metabolites, there were several flunixin metabolites present in the polar and non-polar regions of the HPLC profile of milk that were not structurally identified. While those components occurred in the same chromatographic regions as did flunixin conjugates (polar metabolites) and flunixin methyl ester (a non-polar metabolite) in the rat profiles, efforts were not made to confirm those assignments, in large part because of the small amount of residue present in the milk samples. The lack of identification of these polar and non-polar flunixin metabolites in milk was judged not to be of concern because only traces of those metabolites would be present in milk following the end of the assigned milk discard time (36 hours or three 12-hour milkings). There was also no evidence that those metabolites would be of greater toxicological concern than for parent flunixin.

Table 4.4 Comparison of Flunixin Residues in Milk and Rat Tissues and Excreta

% of Total Radioactive Residue (TRR)											
	Rats								Milk, Post-Final Dose		
	Urine		Feces		Liver		Kidney		First	Second	Third
Metabolite	M	F	M	F	M	F	M	F			
Flunixin	57.1	50	15	14.2	87.1	82.3	91.0	69.0	17.9	20	21.7
4´-OH fln	1.9	1.7	6.6	8.1	NDa	ND	ND	ND	6.3	5.3	9.4
5-OH fln	1.2	7.8	4.7	3.6	0.01	1.7	1.7	ND	46.1	16.9	21.8
2´-MeOH fln	10.2	10.1	11.6	7.8	ND	0.59	0.38	ND	1.3	2.1	1.8
Sulfate conjugate b	3.8	3.5	6	6.6	ND	ND	ND	ND	NTFc	NTF	NTF
Other conjugates d	11.4	12.1	13.8	19.8	ND	ND	ND	ND	NTF	NTF	NTF
fln conjugates	15.2	15.6	19.8	26.4	NAe	NA	NA	NA	NA	NA	NA
Polar f	12.0	12.9	25.2	27.3	NA	NA	NA	NA	8.2	10.3	7.5
Non-polar g											
Fln methyl ester	ND	ND	ND	ND	0.38	0.05	0.46	11.3	NTF	NTF	NTF
Fr-7 (milk)	NA	NA	NA	NA	NA	NA	NA	NA	11.9	23.9	19.8
Fr-8 (milk)	NA	NA	NA	NA	NA	NA	NA	NA	4.7	11.5	9.7
Fr-9 (milk)	NA	NA	NA	NA	NA	NA	NA	NA	2.2	6.0	4.3
Fr-10 (milk)	NA	NA	NA	NA	NA	NA	NA	NA	1.3	3.6	3.6
Fr-12 (rat)	2.3	2.3	6.1	4.7	NA	NA	NA	NA	NA	NA	NA
Fr-13 (rat)	0.9	0.7	4.8	2.8	NA	NA	NA	NA	NA	NA	NA
Fr-14 (rat)	0.6	0.5	2.8	2.2	NA	NA	NA	NA	NA	NA	NA
Total Non-Polar h	3.8	3.5	13.8	9.7	ND	ND	ND	ND	20.2	45.0	37.4

[a] Not detected
[b] Sulfate conjugate of the 4'-OH residue
[c] Not tested for
[d] Acid-labile conjugates of the 5-OH and flunixin residues
[e] Not applicable
[f Sum] of residues eluting before 2'-MeOH flunixin in HPLC analyses, including sulfate conjugate
[g] Residues eluting after flunixin in HPLC analyses including methyl ester of flunixin in rat tissues, Fr-12-Fr-14 in rat excreta, and Fr-7 through Fr-10 in milk. Fr-14 in rat excreta corresponds to retention time of flunixin methyl ester.
[h] Any discrepancies in totals due to rounding.

Results for rats are from SPRI SN 94702 as calculated from Table XXIII for single analyses of composited excreta (% TRR for feces from males is the average of two values) or directly from Tables XXIV-XXVII for single analyses of composited tissues. Results for milk are from SPRI SN 98493, Appendix F (Report Amendment #2), Tables F-1 to F-3. Note that values for first and second milkings are means from 8 animals whereas for the third milking means are from the three animals with the highest residue levels only.

E. TARGET TISSUE, MARKER RESIDUE, LIVER AND MUSCLE TOLERANCES, AND WITHDRAWAL TIME FOR LACTATING DAIRY CATTLE

Residues of flunixin in the edible tissues of lactating cattle are regulated with the same tissue tolerances and slaughter withdrawal time approved for beef cattle as shown below.

target tissue:	liver
marker residue in liver:	unchanged flunixin
tolerance in liver:	0.125 ppm as unchanged flunixin
tolerance in muscle:	0.025 ppm as unchanged flunixin
withdrawal time:	four days after last dose

The assignments of the target tissue, marker residue, tissue tolerances, and withdrawal time listed above are discussed in the FOI summary made available when the beef cattle approval under a supplement to NADA 101-479 was codified on July 20, 1998 (63 FR 38749). No new residue or metabolism studies in tissues of cattle were conducted for the current supplement to NADA 101-479.

F. SELECTION OF MARKER RESIDUE IN MILK

The milk samples from the cows in total residue study 98493 were assayed for total radioactivity then profiled by HPLC radiochromatography for flunixin and its metabolites. The results of those measurements are listed in **Tables 4.1, 4.2,** and **4.4**, and they show that 5-hydroxy flunixin was the most abundant compound in most of the milk samples. That finding and the development of a determinative assay procedure resulted in the 5-hydroxy metabolite of flunixin being chosen as the marker residue in milk.

G. ASSIGNMENT OF THE TOLERANCE IN MILK

The tolerance of 2 ppb for the flunixin marker residue (5-hydroxy flunixin) in milk was assigned on the basis of the marker residue to total residue ratios determined as part of study 98493 (see **Table 4.3)**. The mean marker/total ratio was calculated to be 0.18 for seventeen individual milk samples that had total residue levels in the range of 5 ppb to 20 ppb. Ratios in the range of 5 ppb to 20 ppb ^{14}C radioactivity were chosen for the calculation because those were the milk samples with total residue values near the milk safe concentration of 10 ppb where the tolerance should be determined (see **Section 4.B. Assignment of Safe Concentrations**). The 0.18 ratio was rounded to 0.2, which then gave 2 ppb (0.2 X 10 ppb) as the tolerance.

H. STUDY TO ESTABLISH THE MILK DISCARD TIME

The residue study described below was conducted in lactating dairy cows under field use conditions with Schering-Plough's commercial flunixin product, BANAMINE Injectable Solution. The study provided the milk residue data needed to calculate a discard time for milk from dairy cows treated with flunixin.

Study Title: "SCH 14714: A Final Residue Depletion Study of SCH 14714 (Flunixin)-NMG in Bovine Milk Following IV Administration"

Study No.: 99093

Study Director: Maureen Ngoh, Ph.D., Schering-Plough Research Institute, Lafayette, NJ

Investigators: In-Life Testing Facility:
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Analytical Facility:
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Animals:

Thirty-five Holstein dairy cows from Lone Oak Farms (Hanford, CA) were acclimated for 14 days. From that number, a set of twenty-seven cows was chosen which included the following: Group I, 9 cows with average milk production > 14.4 kg/milking; Group II, 9 cows with average milk production < 14.4 kg/milking but > 8.7 kg/milking; and Group III, 9 cows with average milk production less than 8.7 kg/milking. Two of the 27 cows (1 cow from Group I and 1 cow from Group II) were randomly identified as replacement cows.

Test Article:

The test article, BANAMINE Injectable Solution was the commercial product, which contains 50 mg flunixin as free acid/mL or 83 mg flunixin as N-methyl glucamine (NMG) salt/mL.

Route of Drug Administration and Time/Duration of Dosing:

Each cow was dosed on three successive days via the jugular vein using 3.65 mg flunixin-NMG (2.2 mg flunixin free acid)/kg body weight/day administered 24 ± 3 hours apart. On Days 1 and 3, the left jugular vein was used and on Day 2, the right jugular vein was used for dosing. To ensure intravenous administration

of flunixin·NMG, the syringe was aspirated to draw blood back periodically during dosing. Injections were administered right after the morning milking and approximately 24 hours apart from the previous dose.

Sample Collection:

Control milk was collected in the morning from each cow prior to the administration of the first dose. Milk was collected twice daily (about 12 hours apart) during the three days of treatment and for six days following the last administration of BANAMINE Injectable Solution. Each milking from each cow was separately collected, thoroughly mixed, and prepared for sub-sampling. Frozen sub-samples of milk were shipped by HMS Veterinary Development Incorporated in boxes containing dry ice to ABC Laboratories for analyses.

Residue Depletion in Milk:

The average concentration of 5-hydroxy flunixin measured in the first milking (6/16/99 PM) after the last administration of drug was 20.9 ppb for all cows (**Table 4.5**). In the second milking (24 hours) after the last dose administration, the average concentration of 5-hydroxy flunixin for all cows had depleted to 3.4 ppb. In the fourth milking (48 hours) after the last administration of the drug, the incurred levels of 5-hydroxy flunixin in milk had depleted to values below 0.5 ppb for 24 out of 25 cows. By 72 hrs after the last dosing, the levels of 5-hydroxy flunixin decreased to below 0.5 ppb for all cows.

Table 4.5 Mean Measured Concentrations (ppb) for 5-Hydroxy Flunixin; All Cattle Combined; Three Replicate Values per Milk Sample per Cow.

	Day 1 pm	Day 2 am	Day 2 pm	Day 3 am	Day 3 pm	Day 4 am	Day 4 pm	Day 5 am	Day 5 pm
					Milking 1	Milking 2	Milking 3	Milking 4	Milking 5
					12 hr	PD-1-AM	PD-D-1 PM	PD-D-2 AM	PD-D-2 PM
mean	17.9	2.9	21.3	2.3	20.9	3.4	1.0	1.4	0.6
SD	10.24	2.76	12.11	1.75	8.83	2.42	0.63	0.12	0.014
n	73	71	74	66	75	72	30	3	3

Calculation of Milk Discard Period:

The agency's statistical tolerance limit method (99% tolerance limit with 95% confidence) was used to calculate a milk discard period for flunixin in milk. That procedure used with the residue depletion data in study 99093 and the 2.0 ppb tolerance in milk gave a milk discard period of 36 hours (three milkings if every 12 hours) post final dose.

I. REGULATORY METHOD:

1. Determinative Procedure:

Results for the determinative assay of flunixin in cattle liver are summarized in the FOI summary made available when the NADA was codified on July 20, 1998 (63 FR 38749).

For the determinative assay in milk, 5-hydroxy flunixin is extracted from acidified milk using a solution of acetone/ethyl acetate (1:1). The organic layer is removed after centrifugation of the sample. The acidified sample is extracted three more times with acetone/ethyl acetate (1:1) and the organic extracts are combined. A fraction of the organic extract is further purified on a pre-conditioned strong cation exchange (sulfonic acid (SCX) cartridge) SPE column. 5-Hydroxy flunixin is eluted from the SCX-SPE column using basic methanol. The eluate is concentrated and analyzed using LC/MS/MS in the positive ionization mode. The MS/MS system was optimized to monitor the ionic transition from the precursor ion m/z 313.1 to the product ion m/z 295.1. The method was demonstrated to reliably quantitate 5-hydroxy flunixin residues at levels of 1 ppb to 5 ppb 5-hydroxy flunixin in milk. No interference was observed from 16 veterinary drugs commonly used in cattle.

2. Confirmatory Procedure:

Results for the confirmatory assay of flunixin in cattle liver are summarized in the FOI summary made available when the NADA was codified on July 20, 1998 (63 FR 38749).

The confirmatory method utilizes liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methodology applied to the extract obtained from the determinative method work-up. Confirmation of the presence of 5-hydroxy flunixin was achieved by maximizing the formation of m/z 295 from the parent ion at m/z 313, followed by further ionization of m/z 295 ([M-H_20+H]+) to ions m/z 275 (M+H-H_2O-HF), m/z 252 (M+H-H_2O-CH_3-CO), m/z 226 (M+H-H_2O-H_2F_2-CO-H), and the base peak m/z 280 (M+H-H_2O-CH_3). Any unaltered ions at m/z 295 are also monitored.

3. Display of Method:

The validated regulatory method for the determination and confirmation of residues of flunixin in milk is on file at the Center of Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

V. AGENCY CONCLUSIONS:

The data submitted in support of this supplemental NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that BANAMINE Injectable Solution when administered at the approved dose for beef and dairy cattle is safe and effective for the control of pyrexia associated with bovine respiratory disease, endotoxemia, and acute bovine mastitis; and for the control of inflammation in endotoxemia.

Tolerances are established in 21 CFR 556.286 as follows: 25 ppb in muscle, 125 ppb in liver, 2 ppb in milk. Cattle must not be slaughtered for human consumption within 4 days of the last treatment. Milk that has been taken during treatment and within 36 hours after the last treatment should not be used for food. Not for use in dry dairy cows. A withdrawal time has not been established for use in preruminating calves. Do not use in calves to be processed for veal.

Labeling restricts this drug to use by or on the order of a licensed veterinarian. This decision was based on the following factors: (a) the product contains a nonsteroidal anti-inflammatory 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)(v),(x), & (xii) this is a Category II change, that did not require a reevaluation of the safety or effectiveness data in the parent application.

Under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act , this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval. The three years of marketing exclusivity applies only to the new indication of control of pyrexia associated with acute bovine mastitis. The application contains investigations conducted or sponsored by the applicant that demonstrate substantial evidence of effectiveness for the control of pyrexia associated with acute bovine mastitis.

No patent information was submitted with this application.

VI. ATTACHMENTS:

Facsimile Labeling is attached as indicated below:

A. Package insert

B. 50, 100, and 250 mL bottle labeling

C. 50, 100, and 250 mL carton labeling