FREEDOM OF INFORMATION SUMMARY

1. General Information:

NADA Number:	140-994
Sponsor:	Lloyd, Inc. 604 West Thomas Avenue, P.O. Box A Shenandoah, Iowa 51601, U.S.A.
Trade Name:	Tolazine™ Injection
Established Name:	Tolazoline Hydrochloride, USP, Sterile Solution
Dosage Form:	Sterile injectable solution
How Supplied:	100 mL multiple dose vials
Amount of Active Ingredients:	Each mL of Tolazine contains tolazoline hydrochloride equivalent to 100 mg base activity per mL.
Route of Administration:	Intravenous
How Dispensed:	Prescription (R_X)
Species:	Equine
Recommended Dosage:	The Tolazine dose is 4.0 mg/kg body weight or 1.8 mg/lb (4 mL/100 kg or 4 mL/220 lb) given slowly intravenously.
Pharmacological Category:	alpha-2 adrenoreceptor reversing agent and antagonist
Indications:	Tolazine should be used in horses when it is desirable to reverse the effects of sedation and analgesia caused by xylazine.

2. Effectiveness:

a. Pivotal Efficacy Study #1

1) Type of study: Dose determination study

2) Names and addresses of investigators who conducted the study:

John C. Thurmon, DVM, MS, College of Veterinary Medicine
University of Illinois, Urbana, IL

David Sisson, DVM, College of Veterinary Medicine
University of Illinois, Urbana, IL

3) General design of the investigation:

a) Purpose of the study: The study was designed to test the efficacy and safety of graded doses of tolazoline on ponies treated with tolazoline at 0, 2.0, 4.0, and 8.0 mg/kg body weight, following sedation with xylazine.

b) Test animals: Twelve female and 12 male healthy adult ponies were randomly assigned to 4 test groups.

c) Type of controls: Two types of controls were used:

(1) each animal served as its own control and was evaluated for behavioral and pharmacologic effects before and after injection of tolazoline;
(2) one group received a placebo instead of tolazoline.

d) Diagnosis: Testing was done on healthy animals.

e) Dosage form: Sterile aqueous solution. The product tested was identical to the product to be marketed.

f) Route of administration: Intravenous (IV)

g) Dosages used:

Dose Group	Xylazine IV	Tolazoline IV
0X	1.1 mg/kg	diluent control
0.5 X	1.1 mg/kg	2.0 mg/kg
1X	1.1 mg/kg	4.0 mg/kg
2X	1.1 mg/kg	8.0 mg/kg

Xylazine was administered by a single IV injection into a catheter inserted into a catheter inserted in the jugular vein; tolazoline or diluent control was administered in the same manner 8 minutes later.

h) Test duration:

Tests were conducted during a 24 hour period. Testing and injection times relative to tolazoline injection were as follows:

Measurements were made 6 times on each horse, twice prior to any injections, once after the xylazine injection, and three times after the antagonist injection. Electrocardiograms were conducted 7 times, with the seventh measurement one day after the tolazoline injection.

Treatment and Observations Summary:

Time 1	Time 2	Xylaz.	Time 3	Tolaz.	Time 4	Time 5	Time 6	Time 7
-(60-30)	-(30-10)	-8	-2	0	+5	+35	+120	+1 day

Note: Times are in minutes except for Time 7
ECG Time 4 = +8 min; ECG Time 5 = +30 min

i) Pertinent parameters evaluated:

(1) Sedation
(2) Analgesia
(3) Electrocardiogram (heart rate)
(4) Adverse Signs

The effect on sedation was assessed by head carriage and startle response using a score of 0, 1, 2, or 3 representing degrees of sedation with 0 being alert and 3 being sedated. The effect on analgesia was assessed by needle prick using a similar scoring system, with 0 being no analgesia and 3 being complete analgesia.

4) Results:

a) Sedation:

(1) Head Carriage

Means of Head Carriage Scores (Table 1)

Dose Group	n =	Time B@	Time 3	Time 4	Time 5	Time 6	Group Means
0X	6	0.17	2.33	2.33	1.50	0.17	1.30
0.5X	6	0.00	2.67	2.33	0.67*	0.00	1.13
1X	6	0.00	2.50	1.17*	0.33*	0.00	0.80
2X	6	0.17	2.50	1.00*	0.17*	0.17	0.80
Time Means	24	0.08	2.50	1.71	0.67	0.08	1.01

@Time B (baseline) = (Time 1 + Time 2)/2
*Significance, $p<.05$, of a group at a given time designation by Dunnett test; one-sided comparisons of change from baseline

(2) Startle Response

Means of Startle Response Scores (Table 2)

Dose Group	n =	Time B	Time 3	Time 4	Time 5	Time 6	Group Means
0X	6	1.17	2.00	2.00	1.33	0.83	1.47
0.5X	6	1.25	1.50	2.17	1.50	1.67	1.62
1X	6	0.75	1.33	1.50	0.67	0.33	0.92
2X	6	1.17	2.00	1.33	1.00	1.00	1.30
Time Means	24	1.08	1.71	1.75	1.13	0.96	1.32

b) Analgesia

(1) Needle Prick

Means of Needle Prick Scores (Table 3)

Dose Group	n =	Time B	Time 3	Time 4	Time 5	Time 6	Group Means
0X	6	0.75	2.50	2.33	1.67	1.17	1.68
0.5X	6	1.17	2.50	2.00	1.50	1.17	1.67
1X	6	1.17	2.33	1.50*	1.33	1.17	1.50
2X	6	1.08	2.67	1.67	1.33	1.17	1.58
Time Means	24	1.04	2.50	1.88	1.46	1.17	1.61

*Significance, $p<.05$, of a group at a given time designation by Dunnett test; one-sided comparisons of change from baseline

With reference to tables 1, 2, and 3 ,there were no significant differences between the 1X group (4.0 mg/kg) and the 2X group (8.0 mg/kg) at times 4 and 5. Therefore, the lower dose of 4 mg/kg is appropriate for the recommended dose.

c) Electrocardiogram (heart rate):

Means of Heart Rate (Table 4: beats/minutes)

Dose Group	n =	Time B	Time 3	Time 4	Time 5	Time 6	Time 7	Group Means
0X	6	38.5	42.5	34.0	33.5	37.0	52.0	39.6
0.5X	6	40.3	36.5	33.5	32.5	33.5	50.0	37.7
1X	6	41.8	31.5	36.0	31.5	33.0	62.5	39.4
2X	6	35.8	30.5	42.5*	38.0	32.5	46.0	37.6
Time Means	24	39.1	35.3	36.5	33.9	34.0	52.6	38.6

*Significance, $p<.05$, of a group at a given time designation by Dunnett test

Elevation of heart rate is an undesirable side effect noted at the 2X dose. Since the 1X and 2X doses had similar efficacy, 4.0 mg/kg (1X) is the recommended dose.

d) Side Effects:

Side effects that were reported included pink mucous membranes (evidence of peripheral vasodilation), piloerection, licking of the lips, elevated heart rate, and restlessness. These effects were not severe and dissipated within 30 minutes.

5) Conclusions drawn from the study data:

Tolazoline was effective at intravenous doses of 4.0 and 8.0 mg/kg in reversing the sedative effects of xylazine, and safe at doses from 2.0 to 8.0 mg/kg. Since elevation of the heart rate occurs at 8.0 mg/kg, 4.0 mg/kg is the optimum dose. The Anderson/Nelson linear-plateau, plateau-linear-plateau models were used to support the choice of 4.0 mg/kg as the dose.

b. Pivotal Efficacy Study #2:

1) Type of study: Clinical Field Trial

2) Names and addresses of investigators:

John A. Kasper, DVM
Mark E. Rigby, DVM
Magnolia, Texas

James Andrew Gardner, DVM
Bert Parker, DVM
Salisbury, North Carolina

Anthony J. Prickett, DVM
Cumming, Iowa

Gordon E. Layton, DVM
Stuart J. Burns, DVM
Paris, Kentucky

3) General design of the investigation:

a) Purpose of study:

The purpose of the study was to confirm the efficacy of the tolazoline dose as a reversing agent and antagonist for xylazine in horses and to test its safety under field conditions.

b) Test animals: 102 horses of various age, sex, weight, and breed

c) Two types of controls were used:

(1) each animal was tested for behavioral/pharmacologic effects before and after injection of the Tolazine™;

(2) half of the test animals were administered tolazoline; half were given saline instead of tolazoline.

d) Diagnosis and procedures for sedation:
Testing was done on healthy animals which were given xylazine for procedures indicated for its use (for example, clipping, cleaning prepuce, suturing lacerations). Veterinarians conducting the clinical trials were instructed to administer either intravenous or intramuscular xylazine (AnaSed®, Lloyd Laboratories Division of Vet-A-Mix). The route of xylazine administration was determined using a randomization table. The IM dose of xylazine was 1.0 mg/lb; the IV dose was 0.5 mg/lb.

e) Dosage form: Sterile aqueous solution
The product tested was identical to the product to be marketed.

f) Route of tolazoline administration: IV

g) Dosage used:
Dose used in study was 4.0 mg/kg body weight (1.8 mg/lb)
Tolazine™ injection (4 mL per 100 kg or 220 lb).

h) Test duration:
Each animal was observed for 50-60 minutes.

The testing and injection times relative to the injection of xylazine are given in Table 1 and Table 2.

Table 1.
Intramuscular xylazine, summary of injection times

Time 1	Time 2	Xylazine Injection	Time 3	Tolazoline Injection	Time 4	Time 5
-30 min	-15 min	0 min	+15 min	+30 min	+35 min	+60 min

Table 2.
Intravenous xylazine, summary of injection times

Time 1	Time 2	Xylazine Injection	Time 3	Tolazoline Injection	Time 4	Time 5
-30 min	-15 min	0 min	+6 min	+20 min	+25 min	+50 min

i) Pertinent parameters measured:

(1) Sedation scores (Head Carriage, Startle Response)
(2) Analgesia (Prick response)
(3) Clinical Signs: Heart Rate, Respiration Rate

Head carriage and startle response were assessed by using a score of 0, 1, 2 or 3 representing levels of sedation with 0 being alert and 3 being sedated. The effect on analgesia was assessed by needle prick using a like scoring system with 0 being no analgesia and 3 being complete analgesia. Heart and respiratory rates were measured in beats and breaths per minute, respectively.

4) Results:
102 cases were used to evaluate the drug. The results are listed in Tables 3, 4, 5, 6 and 7.

a) Sedation:

(1) Head Carriage

Table 3.
Least squares means of head carriage scores combining xylazine routes of administration

Drug	n	TB*	T3	T4	T5
Saline	51	0.001	2.367	1.738	0.539
Tolazoline	51	0.011	2.373	0.889**	0.167**

*TB = average baseline value (Time 1 + Time 2 divided by 2)
**p <.001 (differences between saline and tolazoline)

(2) Startle Response

Table 4.
Least squares means of startle response scores combining xylazine routes of administration

Drug	n	TB	T3	T4	T5
Saline	51	0.633	2.178	1.680	0.978
Tolazoline	51	0.485	2.248	1.339	1.152

b) Analgesia:

(1) Needle Prick

Table 5.
Least squares means of needle prick scores combining xylazine routes of administration

Drug	n	TB	T3	T4	T5
Saline	51	1.320	2.572	2.107	1.548
Tolazoline	51	1.121	2.566	1.752*	1.349

*p <.05 (differences between saline and tolazoline)

c) Clinical Signs:

(1) Heart Rate

Table 6.
Least squares means of heart rate in beats/minute combining xylazine routes of administration

Drug	n	TB	T3	T4	T5
Saline	51	47.5	35.4	37.2	40.1
Tolazoline	51	48.7	34.5	39.5	40.4

(2) Respiration Rate

Table 7.
Least squares means of respiratory rate in breaths/minute combining xylazine routes of administration

Drug	n	TB	T3	T4	T5
Saline	51	21.1	13.6	14.1	15.0
Tolazoline	51	20.1	12.1*	16.3	18.5**

*p <.05 (differences between saline and tolazoline)
**p <.001 (differences between saline and tolazoline)

5) Conclusions drawn from the study:
Tolazoline at 4 mg/kg (1.8 mg/lb) of body weight effectively reversed the sedation and analgesia that were induced by xylazine.

6) Adverse effects reported were minimal and not unexpected. The effects noted were injected mucous membranes of the conjunctiva and pink muzzle in two animals (evidence of peripheral vasodilation), and piloerection in three other horses.

3. Animal Safety:

a. Pivotal Safety Study #1:

1) Type of study: Multiple dose Target Animal Safety (TAS) study evaluating tolazoline only.

2) Names and addresses of investigators:

The study was conducted at the College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

The Study Director was John C. Thurmon, DVM, MS, Professor and Head of Anesthesiology.

Cardiology was evaluated by Dave Sisson, DVM, Diplomate, American College of Veterinary Internal Medicine.

Clinical pathology was done by Joseph L. Dorner, DVM, PhD, Professor, Clinical Pathologist.

Necropsy and pathologic examinations were conducted by Ralph Bunte, DVM, Diplomate, American College of Veterinary Pathologists.

Statistical analyses were conducted by Joseph W. Denhart, DVM, MS, LLOYD, Inc.

3) General design of the investigation:

(a) Purpose of the study:
The study was designed to test the safety of tolazoline in healthy ponies treated at 0, 1X, 3X, and 5X the intended dose.

(b) Test animals:
12 female and 12 male adult ponies, randomly divided into 4 groups

(c) Dosage Form: sterile aqueous solution
The product tested was identical to the product to be marketed.

(d) Doses used:

Group	Dose	Tolazoline
1	0X	diluent control
2	1X	4.0 mg/kg
3	3X	12.0 mg/kg
4	5X	20.0 mg/kg

(e) Route of administration: IV

(f) Test duration:
Each dose was administered through a catheterized jugular vein three times at six hour intervals.

(g) Statistical Considerations:

Statistical analyses were conducted by Repeated Measures Analyses using SYSTAT, MGLH module. ANOVAs were conducted at each Time and the groups compared by the post hoc tests of DUNNETT test and TUKEY HSD Multiple Comparison test.

(h) Parameters measured:

(1) Clinical signs:

Clinical observations for adverse signs were made at -1 day, -60 min, -30 min, 0 min (tolazoline injection #1), +360 min (injection #2), and +720 min (injection #3). Ponies were also monitored closely during the hour following each injection. Monitoring for adverse clinical signs continued daily for 7 days following the drug trial.

(2) Hematology:

Blood samples were collected at -(3 to 10 days), -1 day, +1 day, +3 day, and +7 days. The following parameters were evaluated: PCV,Hb, plasma protein, and WBC with differential.

(3) Serum biochemistry:

Blood samples were collected at -(3 to 10 days), -1 day, +1 day, +3 day, and +7 days. The following parameters were evaluated: BUN, GGT, AST, CPK, glucose, calcium, phosphorus, sodium, potassium, and chloride.

(4) Electrocardiogram (ECG):

ECG were scheduled relative to the first tolazoline injection at -60 to -30 min, -30 to -10 min, +6 to +8 min, and +360 min (immediately prior to second injection).

(5) Necropsy and Histopathology:

One male and one female pony each were necropsied from the control group (0X) and the 5X group on day 7. Histopathology was performed on adrenal glands, kidney, liver, and any grossly abnormal tissues.

4) Results:

(a) Clinical Signs:

Clinical observations for adverse signs provided a clear picture of the physiological response to tolazoline without prior xylazine treatment. Side effects included evidence of peripheral vasodilitation (bright pink mucous membranes), licking and flipping lips, piloerection, clear lacrimal and nasal discharge, sweating, and muscle fasciculation. An apparent increase in gastrointestinal motility was demonstrated by signs of abdominal discomfort and increased defecation or tenesmus with flatulence. These signs were of greater intensity with increasing doses and many of these signs increased in intensity with subsequent doses. All noted signs were transient in nature.

(b) Hematology:

Hematology results were unremarkable.

(c) Serum biochemistry:

Serum biochemistry results were unremarkable.

(d) Electrocardiogram (ECG):

A dose related response was shown in that the control group and the 1X group had no ECG abnormalities, but the 3X and the 5X groups showed tachycardia and cardiac conduction disturbances (evidenced by increased QRS waves and decreased QT waves). The changes did not persist.

The Dunnett test was used to compare the control group (0X) to each of the treatment groups (1X, 3X, and 5X). At $p<.05$, group differences were demonstrated at +6 to +8 minutes after tolazoline administration in the 3X and 5X treatment groups for increased heart rate and in the 5X group for increased QRS wave and decreased QT wave durations.

(e) Necropsy and Histopathology:

Routine necropsy and histopathology failed to detect any treatment related changes.

5) Conclusions based on study data:

Side effects associated with the administration of tolazoline at 4.0, 12.0, and 20.0 mg/kg body weight are dose related and transient. Cardiac and gastrointestinal data demonstrate the main safety concerns associated with the use of tolazoline when given a total of three doses at six hour intervals. Doses of 3X or greater in healthy horses are of questionable safety due to tachycardia and cardiac conduction disturbances.

b. Pivotal Safety Study #2:

1) Type of study: Multiple dose Target Animal Safety (TAS) study evaluating tolazoline following administration of xylazine.

2) Names and addresses of investigators:

The study was conducted at the College of Veterinary Medicine, Iowa State University, Ames, Iowa, by Veterinary Resources, Inc., Ames, Iowa.

The Study Director was David P. Carter, DVM, President of Veterinary Resources, Inc., Ames, Iowa.

The cardiology was evaluated by Wendy A. Ware, DVM, MS, Diplomate ACVIM (Cardiology), Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Clinical pathology was conducted by the Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa, under the direction of Clinical Pathologist, Wayne A. Hagemoser, DVM, PhD, DACVP.

Necropsy and pathologic examinations were done by Joseph S. Haynes, DVM, PhD, DACVP, and additional histopathologic consultations were provided by Ronald K. Myers, DVM, PhD, DACVP, and Lyle D. Miller, DVM, PhD, DACVP, Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

The study was conducted in compliance with the good laboratory practice regulations set forth in 21 CFR Part 58 and the Quality Assurance Unit was under the direction of Phillip Buckler, ABC Laboratories, Inc., Columbia, Missouri.

3) General design of the investigation:

(a) Purpose of the study:

The study was designed to test the safety of tolazoline in horses treated at 0, 1X, 3X, and 5X the tolazoline dose following xylazine administration.

(b) Test animals:

12 female and 12 male adult horses, randomly divided into 4 groups.

(c) Dosage form:

Sterile aqueous solution. The product tested was identical to the product to be marketed.

(d) Dosage Table:

Group	Xylazine (IV)	Tolazoline (IV)
0X	1.1 mg/kg	0 mg/kg
1X	1.1 mg/kg	4.0 mg/kg
3X	1.1 mg/kg	12.0 mg/kg
5X	1.1 mg/kg	20.0 mg/kg

(e) Route of administration: IV

(f) Test duration:

Each horse was acclimated for 14 days, administered the prescribed dosages three times at 24 hour intervals, and evaluated for a seven day post-trial period.

(g) Study schedule:

During the 3 day drug trial, clinical observations were made at the following times:

Time 1: 60 to 30 min before xylazine
Time 2: 10 min after xylazine (10 min before tolazoline)
Time 3: 5 min after tolazoline
Time 4: 20 min after tolazoline
Time 5: 60 min after tolazoline
Time 6: 2 hr after tolazoline

Physiological parameters were measured according to the following schedule:

Time 1: 60 to 30 min before xylazine
Time 2: 10 to 5 min before tolazoline
Time 3: 2 min after tolazoline
Time 4: 5 to 10 min after tolazoline
Time 5: 60 min after tolazoline

(h) Experimental parameters:

(1) Clinical Observations
(2) Electrocardiogram
(3) Heart Rate
(4) Blood Pressure
(5) Respiratory Rate
(6) Hematology
(7) Serum biochemistry
(8) Urinalysis
(9) Necropsy
(10) Histopathology
(11) Weight

4) Results:

Two tolazoline related deaths occurred immediately following administration of the 5X dose of tolazoline (see necropsy results and conclusions discussed below).

(a) Clinical Observations:

(1) Mucous membrane color:

Nearly all horses showed injected mm (increased peripheral vasodilation) after administration of tolazoline at all dose levels. Mucous membranes were normal in all horses within 24 hours.

(2) Piloerection was observed in two horses after receiving tolazoline.

(3) Lip flicking and licking:

Hyperalgesia of the lips was observed in two horses after receiving tolazoline.

(4) Sweating:

Sweating was seen in most horses that received xylazine/placebo and xylazine/tolazoline. It was more intense and lasted longer for horses treated with xylazine/tolazoline.

(5) Lacrimal and nasal discharge:

Nasolacrimal discharge was observed in xylazine/placebo horses and in xylazine/tolazoline horses. The side effect was not tolazoline dose related.

(6) Muscle fasciculation was seen in three horses (one in the xylazine/placebo group and two in the xylazine/tolazoline groups)

(7) Increased defecation was seen more frequently in horses that received tolazoline compared to the control group (not dose related).

(8) Colic and diarrhea:

One horse in the 3X group experienced mild, transient colic and fluid stool on the second and third days of the trial. No treatment was required.

(9) Gastrointestinal motility:

Most horses experienced xylazine-induced hypomotility. Within five minutes of tolazoline administration in many horses, intestinal motility was scored as normal compared to the control group. Two horses in the 3X group experienced gastrointestinal hypermotility, one of which colicked. The hypermotility resolved more quickly on day one compared to the second and third days in the trial (see table).

Gastrointestinal hypermotility seen in 2 horses (3X):

Horse	Day 1	Day 2	Day 3
#95 (colicked)	normal by 1 hr post-tolazoline	normal by 24 hr post-tolazoline	normal by 24 hr post-tolazoline
#91	normal by 1 hr post-tolazoline	normal by 1 hr post-tolazoline	normal by 1 hr post-tolazoline

(b) Electrocardiogram (ECG):

Prolongation of the QRS complex occurred immediately after dosing in four of six horses in the 3X tolazoline group and in all four of the surviving horses in the 5X tolazoline group. ECG measurements after 5X tolazoline administration could not be obtained from the two horses that died. However, tolazoline related cardiac conduction disturbances were the most likely cause of death (see conclusions below).

The following table summarizes the length of the QRS complex in seconds at the five measurement times (each value is the mean of the three injection interval values from each of three days):

Dose	Time 1	Time 2	Time 3	Time 4	Time 5
0X	0.116	0.116	0.117	0.116	0.114
1X	0.108	0.107	0.116	0.117	0.113
3X	0.115	0.116	0.139	0.140	0.129
5X	0.113	0.111	0.149	0.151	0.127

(c) Heart Rate (HR):

The HR in the following table are the means of three injection intervals (one per day):

Dose	Time 1	Time 2	Time 3	Time 4	Time 5
0X	41.9	36.3	32.7	33.4	46.6
1X	39.0	39.6	47.1	36.7	39.0
3X	40.3	36.7	65.7	59.7	39.8
5X	41.9	34.7	71.4	69.2	43.0

Transient tachycardia occurred after tolazoline administration at 1X, 3X, and 5X doses. The degree and duration of tachycardia is increased at 3X and 5X and is dose related (5X > 3X).

(d) Blood Pressure (BP):

The following table represents the mean systolic BP of the three injection intervals at the five measurement times:

Dose	Time 1	Time 2	Time 3	Time 4	Time 5
0X	90.4	94.0	94.3	90.1	76.9
1X	94.0	90.1	113.1	126.6	106.8
3X	91.4	93.7	123.7	132.1	119.6
5X	89.6	102.9	106.0	126.0	143.6

Horses in the 0X group (xylazine only) had BP below the referenced normal value by time 5 on all three days. None of the horses that received tolazoline had BP below the referenced norms at time 5 on any of the three injection days. Tolazoline helped reverse xylazine-induced hypotension.

Some horses experienced hypertension after receiving tolazoline. The highest values were seen in the 3X and 5X horses at times 3 and 4 on day 1

(elevated systolic BP ranged from 148 to 240 mm Hg). On days 2 and 3, all 5X horses (4 horses) showed an initial decline in BP quickly followed by increased BP by 5-10 minutes post-tolazoline. Fewer horses became hypertensive on days 2 and 3, after the initial transient decline in BP.

Only one horse in the 1X dose group exhibited persistent hypertension (on day 3 at time 5). BP returned to normal by 3 hours post-tolazoline. BP for this horse was within the normal reference range on days 1 and 2 after tolazoline administration.

(e) Respiratory Rate (RR):

RR generally decreased following xylazine administration. Tolazoline raised RR back in some horses to prexylazine rates. In others, administration of tolazoline did not have an effect on RR. One horse in the 1X dose group showed a unique increase in RR to approximately twice baseline following each treatment. RR returned to the pretolazoline RR within one hour after treatment.

(f) Hematology and Serum Biochemistry:

The hematology and serum biochemistry results did not identify any safety concerns associated with the administration of xylazine followed by tolazoline.

(g) Urinalysis:

The urinalyses results did not identify any safety concerns associated with the administration of xylazine followed by tolazoline.

(h) Necropsy:

Two mortalities occured immediately after administration of tolazoline at the 5X dose to horses that had received a 1X dose of xylazine. These two horses were completely necropsied. The cause of death could not be determined from gross findings or histopathology alone. However, diffuse congestion coupled with the lack of other necropsy findings and the clinical history, suggested that effects on the cardiac conduction system contributed to death and that these effects were probably due to a 5X dose of tolazoline.

(i) Weight:

Changes in weight did not correlate with dose group and were not considered significant.

5) Conclusions:

The major adverse reaction clinically observed during the study was the death of two horses that received a 5X dose of tolazoline following administration of xylazine. The four surviving horses in the 5X dose group and four (of six) horses in the 3X dose group showed prolonged QRS waves immediately after receiving tolazoline. Prolongation of the QRS complex can predispose to ventricular arrhythmias and possibly death. No ventricular arrhythmias were recorded during the study; however, no ECGs were recorded immediately before the horses died. Transient tachycardia occurred after tolazoline administration at the 1X, 3X, and 5X doses.

Based on tachycardia, ECG conduction disturbances, and negative necropsy findings, ventricular arrhythmia caused by an overdose of tolazoline is the most likely cause of death.

Therefore, a single dose of 1X or 2X tolazoline is safe in clinically normal horses. Doses of 3X or higher cannot be considered safe in healthy horses, due to tachycardia, cardiac conduction disturbances and death.

Gastrointestinal side effects were much more pronounced when tolazoline was administered alone to healthy horses (see safety study #1). Gastrointestinal side effects were much less frequent and less severe in the TAS study using xylazine and tolazoline. As long as tolazoline is always administered after xylazine, these effects do not represent a safety concern in healthy horses at the 1X dose. Doses of 3X or higher are of questionable gastrointestinal safety (one horse with colic and persistent hypermotility).

Tolazoline reversed xylazine-induced hypotension with a mild hypertension resulting in some horses. Hypertension or hypotension related to the use of tolazoline in healthy horses was not a safety concern in this study.

Other clinical signs (injected mucous membranes, piloerection, hyperalgesia of the lips, sweating, lacrimal and nasal discharge, and muscle fasciculation) associated with the use of tolazoline were observed to occur less frequently and less intensely when tolazoline was administered after xylazine (see TAS study results using tolazoline alone). These side effects do not represent a safety concern in healthy horses at doses up to 5X.

4. Corroborative Study:

a. Type of study: Pharmacokinetic Study

b. Names and addresses of investigators:

The study was conducted at the College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

The Study Director was John C. Thurmon, DVM, MS, Professor and Head of Anesthesiology.

Richard F. Bevill, DVM, PhD, Professor, Veterinary Pharmacology, University of Illinois, Urbana, Illinois conducted the sampling, chemical analyses and statistical analyses.

c. General design of the investigation:

1) Purpose of the study:
The study was designed to determine the pharmacokinetics of tolazoline in horses.

2) Test animals: 12 female and 12 male adult ponies were randomly divided into 4 groups for the Target Animal Safety (tolazoline alone) study. During that study, blood samples were obtained from the 1X and 5X ponies for pharmacokinetic evaluation.

3) Dosage form: sterile aqueous solution
The product tested was identical to the product to be marketed.

4) Doses:

Dose	Tolazoline
1X	4.0 mg/kg
5X	20.0 mg/kg

5) Route of administration: Intravenous
Each dose was administered through a jugular vein catheter.

6) Test duration: Samples were obtained during the twelve hours following tolazoline treatment.

7) Experimental Parameters:

The concentration of tolazoline in equine plasma was measured at 0, 3, 6, 12, 18, 30, 60, 120, 240, and 360 minutes after dosing.

d. Results:

A two compartment open model was fit to the plasma concentration versus time profiles for the low doses whereas a three compartment open model was used to fit the plasma concentration versus time profiles for the high doses.

Statistical analyses were conducted by the Autoan computer program (Sedman and Wagner, 1976) and the PC Non-Lin iterative computer program (Statistical Consultants, 1986). The
volume of distribution and total body clearance were calculated using standard formulae (Gibaldi and Perrier, 1982).

For the 4.0 mg/kg dose group, the elimination half-life was one hour; for the 20 mg/kg dose group, the elimination half-life was 1.7 hours.

e. Conclusions drawn from the study data:

Tolazoline was rapidly eliminated from the body in healthy ponies when no other drug was administered.

5. Human User Safety:

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

This drug is for use in horses only and not for use in food-producing animals.

b) Adequate warning statements concerning the accidental administration of tolazoline to the human user are contained in the labeling.

6. Agency Conclusions:

The data submitted in support of this NADA comply with the requirements of Section 512 of the Act and Section 514.111 of the implementing regulations. The data demonstrate that Tolazine (tolazoline hydrochloride), when used under its labeled conditions of use, is safe and effective.

Tolazoline is labeled for use only after xylazine hydrochloride administration as a xylazine antagonist. Xylazine is a prescription drug used by licensed veterinarians to induce sedation and analgesia. Accordingly, tolazoline hydrochloride is a prescription new animal drug.

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for 5 years of marketing exclusivity beginning on the date of approval because no active ingredient (including any ester or salt of the active ingredient) of the drug has been approved in any other application.

7. Labeling:

a. Package Insert

b. 100 mL vial label

c. Carton label