DRAFT FINAL REPORT

Pilot Safety Study of Escalating Doses of 7-Hydroxy Mitragynine and Pseudoindoxyl Mitragynine Administered Daily for 7-day Intervals to Mature Dogs

STUDY NUMBER: **MGN-25-001**

SPONSOR: KanPro Research, Inc
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INVESTIGATOR: Craig R. Reinemeyer, DVM, PhD
East Tennessee Clinical Research

TESTING FACILITY: East Tennessee Clinical Research, Inc.
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Study Initiation Date: 05MAR25
Experimental Start Date: 10MAR25
Experimental End Date: 19MAY25

APPROVAL SIGNATURES:

Investigator: _____ _____
Craig R. Reinemeyer, DVM, PhD Date:

Sponsor Representative: _____ _____
Philip Gao, PhD Date

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1.0 TITLE

Pilot Safety Study of Escalating Doses of 7-Hydroxy Mitragynine and Pseudoindoxyl Mitragynine Administered Daily for 7-day Intervals to Mature Dogs

2.0 PROTOCOL NUMBER

2.1 SPONSOR STUDY NUMBER

PRx2025-01

TESTING FACILITY NUMBER:

MGN-25-001

3.0 SUMMARY

4.0 JUSTIFICATION

Various formulations of mitragynine (Kratom) are currently marketed over-the-counter and used by human patients for treatment of pain. In preparation for filing an INADA and/or IND application with the U.S. Food and Drug Administration, the sponsor desires to evaluate the safety of two mitragynine products at doses higher than those expected to be used clinically. The first product is 7-Hydroxy Mitragynine (7-HMG), an oxidative metabolite of mitragynine, the most abundant alkaloid in the leaves of *Mitragyna speciosa*. 7-HMG is further metabolized to Pseudoindoxyl Mitragynine in the plasma. Both metabolites are potent and full μ opioid receptor (MOR) agonists. A pharmacokinetic and safety study of 7-HMG, administered as a single dose, was performed in Beagle dogs (ref). It was found that an oral dosage of 1 mg/kg 7-HMG was well-tolerated with no observed adverse events or significant changes to clinical laboratory tests. Given the opioidergic potency of mitragynine metabolites, a pilot safety study of escalating doses was performed in Beagle dogs using both 7-HMG and Pseudoindoxyl mitragynine.

5.0 STUDY OBJECTIVE

The objective of the study was to evaluate the safety of two of KanPro's proprietary oral solution formulations of a candidate therapeutic product in escalating doses (1, 2 and 4 mg/animal) administered orally for 7-day intervals to mature dogs. The safety of either product at all doses was evaluated by comparison to control dogs treated contemporaneously with an oral placebo solution (saline). Systemic and local safety were evaluated by hematologic and clinical

chemistry parameters, and by scheduled clinical observations of behavior, health and regular physical examinations.

6.0 STUDY LOCATION

East Tennessee Clinical Research, Inc.
80 Copper Ridge Farm Rd.
Rockwood, TN 37854
865.354.8420

7.0 PERSONNEL

7.1 SPONSOR REPRESENTATIVE

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7.2 INVESTIGATOR

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7.3 ATTENDING VETERINARIAN

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8.0 INVESTIGATIONAL VETERINARY PRODUCTS (IVPs)

8.1 INVESTIGATIONAL VETERINARY PRODUCT (IVP) #1

Generic Name	7-OH-mitragynine
Trade Name	*TBD*
Active ingredients	7-OH-mitragynine
Inactive Ingredients	Vegetable glycerin, ascorbic acid, water
Dosing form	Oral solution
Dose(s) to be tested	1 mg SID Days 0 to 6; 2 mg SID Days 7 to 13; 4 mg SID Days 14 to 20
Manufacturing site	KanPro, Lawrence, KS
Lot Number	To be included in the Final Report
Expiration/ Retest Date	To be included in the Final Report
Packaging	To be described in the Final Report
Drug storage during study	Glass amber vial, 4°C
Purity/ Potency of the Product	98.0%; 10.0-50.0 mg/mL
Material Safety Data Sheet	None available. Personnel handling or administering the IVP wore PPE appropriate for the task.
Stability data	To be described in the Final Report

8.2 INVESTIGATIONAL VETERINARY PRODUCT (IVP) #2

Generic Name	Pseudoindoxyl mitragynine
Trade Name	*TBD*
Active ingredients	Pseudoindoxyl mitragynine
Inactive Ingredients	Vegetable glycerin, water
Dosing form	Oral solution
Dose(s) to be tested	1 mg SID Days 0 to 6; 2 mg SID Days 7 to 13; 4 mg SID Days 14 to 20
Manufacturing site	KanPro, Lawrence, KS
Lot Number	To be included in the Final Report
Expiration/ Retest Date	To be included in the Final Report
Packaging	To be described in the Final Report
Drug storage during study	Glass amber vial, 4°C
Purity/ Potency of the Product	96.0%; 10.0-50.0 mg/mL
Material Safety Data Sheet	None available. Personnel handling or administering the IVP wore PPE appropriate for the task.
Stability data	To be described in the Final Report

8.3 CONTROL VETERINARY PRODUCT (CVP)

Generic Name	Saline solution
Trade Name	*TBD*
Active ingredients	0.9% Sodium chloride solution
Inactive Ingredients	*TBD*
Dosing form	Oral solution
Dose(s) to be tested	Equivalent in volume to the highest dose administered to any Group 1 or 2 dog during the corresponding 7-day treatment period
Manufacturing site	TBD
Lot Number	To be included in the Final Report
Expiration/ Retest Date	To be included in the Final Report
Packaging	To be described in the Final Report
Drug storage during study	Clear glass vial, ~25°C
Purity/ Potency of the Product	0.9%; 9 mg/mL
Material Safety Data Sheet	Download from the internet.
Stability data	Stipulated by presence of an expiration date

8.4 DRUG STORAGE DURING STUDY

The CVP (saline) was stored in a locked cabinet and maintained at room temperature. IVPs #1 and #2 were stored at 4°C in an amber-colored glass vial.

8.5 INVESTIGATIONAL VETERINARY PRODUCT ACCOUNTABILITY

Records of the receipt, distribution, storage, and disposition of test materials were documented on the *Investigational Veterinary Product Receipt and Accountability Record (IVP #1 and IVP #2)*. Similar details regarding the saline placebo were

documented on the *Control Product Receipt and Accountability Record*. At the conclusion of the study, all test materials, including empty, full or partially full containers of IVP, were returned to the Sponsor or disposed on-site at the direction of the Sponsor.

9.0 STUDY DESIGN

9.1 STUDY DESIGN SUMMARY

This was a randomized, masked, pilot study to evaluate the safety of two mitragynine compounds in proprietary oral formulations. Mitragynine is an active pharmaceutical ingredient under investigation by American Shaman as a candidate treatment for pain in animals and humans. A minimum of 18 healthy, mature Beagle dogs were acclimated to study conditions for at least seven days prior to randomization and allocation to treatment groups, and initial administration of the experimental treatments. Candidates were healthy, as determined by daily general health observations, a physical examination, and baseline hematology, urinalysis and clinical chemistry analyses during the acclimation period. Candidates meeting inclusion criteria were allocated randomly to one of three treatment groups as presented in Table 2. Doses of the assigned product were administered beginning on Day 0. The safety of both compounds and all dose levels were evaluated by comparison of clinical parameters and urinalysis (weekly) and hematologic and clinical chemistry values at biweekly intervals post-treatment. The previous weekly dose was doubled in each of three successive treatment periods. After study completion, laboratory results and clinical observations were compared among groups to identify any potential adverse signs attributable to administration of the mitragynine compounds. After termination of the study on Day 20, dogs were returned to the facility colony and managed in compliance with testing facility SOPs.

9.2 TREATMENT GROUPS

Sixteen mature dogs meeting inclusion criteria were randomly assigned to one of the following treatment groups:

Table 1. Description of Treatment Groups

Treatment Group	No. of dogs	Treatment	Doses Administered		
			Days 0 – 6	Days 7 – 13	Days 14 - 20
Group 1	6	7-OH MGN	10 mg	20 mg	40 mg
Group 2	6	Pseudo-indoxyl MGN	10 mg	20 mg	40 mg
Group 3	4	Placebo (Saline)	Equivalent to volumes for Groups 1 and 2		

9.3 RANDOMIZATION AND MASKING PROCEDURES

On or before Day -1, 16 mature Beagles (8 male; 8 female) meeting inclusion criteria were blocked by gender, and within each block dogs were ranked by order of decreasing body weight. Of the six heaviest male subjects, each three consecutively ranked dogs comprised a replicate. Three identical objects were each labeled with a number corresponding to a treatment group (Table 1) and placed into an opaque

container. Starting with the heaviest male, one object was selected blindly from the container, and the code number thereupon constituted that dog's treatment group assignment. This process was continued for the remaining two dogs in the replicate as the labeled objects were withdrawn from the container, one at a time and without replacement. The three objects were replaced in the opaque container and the process repeated for replicate #2. For the remaining two candidates (the lightest males), object #3 was removed (due to unequal sizes of treated vs. control groups) and the process repeated for allocation to Groups 1 or 2. This randomization scheme resulted in the allocation of three male dogs to Group 1, three to Group 2 and two males to control Group 3 (eight total).

The randomization and allocation process was then repeated identically for allocation of the eight female candidates.

10.0 STUDY SCHEDULE

10.1 SCHEDULE OF EVENTS

Table 2. Schedule of Events

Study Day(s)	GHOs[1, 2]	TX (BID)	Blood Sample	Study Activities
Prior to Day 0	X		X	Begin Acclimation period. Physical Examination Collect baseline CBC, Clinical Chemistry and Urinalysis Randomization and Allocation Body Weights
Day 0		X, X		Week 1 Treatment: 10mg/kg Clinical Health Observations
Day 1 and 2	X, X	X, X		
Day 3	X, X	X, X	X	Collect CBC and Clinical Chemistry
Day 4 and 5	X, X	X, X		
Day 6	X, X	X, X	X	Physical Examination Collect CBC, Clinical Chemistry, and Urinalysis, Body Weights
Day 7		X, X		Week 2 Treatment: 20mg/kg Clinical Health Observations
Day 8 and 9	X, X	X, X		
Day 10	X, X	X, X	X	Collect CBC, Clinical Chemistry, and Urinalysis
Day 11 and 12	X, X	X, X		
Day 13	X, X	X, X	X	Physical Examination Collect CBC, Clinical Chemistry, and Urinalysis, Body Weights
Day 14		X, X		Week 3 Treatment: 40mg/kg Clinical Health Observations
Day 15 and 16	X, X	X, X		

Study Day(s)	GHOs[1, 2]	TX (BID)	Blood Sample	Study Activities
Day 17	X, X	X, X	X	Collect CBC, Clinical Chemistry, and Urinalysis
Day 18 and 19	X, X	X, X		
Day 20	X, X	X, X	X	Physical Examination Body Weights Collect CBC, Clinical Chemistry, and Urinalysis
Day 21	X, X	X, X		Study Termination

[1] A.M and P.M observations will be conducted at least 6 hours apart

[2] Vomiting Observations will be conducted at 15 minutes post treatment (±5min) for each treatment

10.2 DATE OF INITIATION

05MAR2025

10.3 PROPOSED DATE OF COMPLETION

Date when the Investigator signs the Final Study Report.

11.0 ANIMAL SELECTION AND IDENTIFICATION

11.1 SOURCE OF DOGS

Candidate dogs were purchased from Ridglan Farms, Mt. Horeb, WI 53572 and transported to the testing facility.

11.2 DOG DEMOGRAPHICS

Characteristics of animals to be enrolled in the study are presented in Table 3.

Table 3. Characteristics for Animal Selection

Breed	*Canis familiaris*, purpose-bred Beagle
Age	>6 mos. of age on Day 0
Sex	Intact males and females
Weight	Appropriate for a healthy Beagle for its age and sex
Number	16 in total for treatment; 8 males and 8 females
Health	In good health, based on daily observations and a physical examination conducted during the acclimation period
Temperament	Dogs must be tolerant of study procedures

11.3 DOG IDENTIFICATION

Dogs were uniquely identified by a permanent tattoo inside the right pinna and by a cage card presenting the dog I.D. and gender.

11.4 INCLUSION / EXCLUSION CRITERIA

For enrollment in the study, each candidate had to meet all the criteria listed in Table 3. Reasons for inclusion or exclusion were documented on the *Inclusion / Exclusion Statement*. The two excluded candidates were one male and one female with the smallest body weight.

12.0 ANIMAL MANAGEMENT AND HOUSING

12.1 HOUSING

Dogs were housed in individual ~4 ft. X 4 ft. X 3.5 ft. suspended cages constructed of galvanized metal panels. Each cage was equipped with individual food and water containers, as well as a 24" X 24", solid, polypropylene resting platform. The test facility provided appropriate space allocation, shelter, sanitation, feeding, watering, etc. as dictated by facility standard operating procedures. Cages were located in a climate-controlled room.

Dogs experienced a photoperiod of ~12 hours light and ~12 hours of darkness, the former provided by overhead fluorescent fixtures. The light switch was controlled by an electronic timer. Brief changes to this lighting program could be made in order to accommodate study procedures.

Heating and cooling were electronically controlled and were set to maintain the animal room in a temperature range from 64 to 84ºF. Relative humidity was monitored daily, and HVAC equipment was adjusted to target 30% to 70% relative humidity. Environmental conditions were documented daily on the *Environmental Monitoring Record*.

12.2 DIET

A commercial dry dog food (*i.e.*, LabDiet® 5LI8 High Density Canine Diet, PMI® Nutrition International, Inc.) was provided in quantities consistent with the manufacturer's recommendations for the size and age of the animal. The quantity of food provided was based on the intent to support maintenance and growth. Feed labels presenting the guaranteed product analysis of all foodstuffs are included in the raw data.

Dogs were fed twice daily in individual, stainless steel bowls or buckets, and feeding activities will be documented on the *Canine Husbandry Record*. Contaminants that might confound study objectives were not expected, so no food samples were collected or stored for potential future analysis.

To generate additional information regarding the effects of prandial status on mitragynine tolerability, it was proposed that A.M. doses be administered on an empty stomach, but that P.M. doses were administered approximately 1 hour after feeding.

12.3 WATER

Water was sourced from a local, public utility. Water was available *ad libitum* and was provided in stainless steel, 2 L pails that were cleaned once daily and filled twice daily. No contaminants that might impact the outcomes of the study were expected, so no samples of water were collected or retained for potential analysis.

12.4 CLEANING

Holding cages were cleaned at once daily and sanitized with a commercial disinfectant at least once biweekly. Dogs were removed from their primary housing to facilitate cleaning activities.

12.5 FACILITY DIAGRAM

A diagram of the animal facility, including caging details and placement of food and water containers and resting platforms, was included in the final report.

12.6 CONCOMITANT THERAPY AND/OR MEDICATIONS

Other than protocol specific treatments, the only veterinary drug administered to dogs from Day -7 (start of acclimation) until Day 20 (study termination) was an EarMed Boracetic flush for an interdigital cyst on study dog ALL-4. The flush was poured onto a gauze square and then applied to the interdigital cyst on 05MAY25. Details of boracetic flush administration were recorded on the *Concomitant Medication Record.*

13.0 STUDY PROCEDURES

13.1 ACCLIMATION

Candidates underwent an extended acclimation period (51 days) due to delays in the IVP manufacturing process. During this entire period, diet, water, environment and management simulated the conditions expected during the in-life phase of the study. No medications or vaccinations were permitted after initiation of the acclimation period or thereafter for the duration of the study.

13.2 GENERAL HEALTH OBSERVATIONS

Animal observations were performed twice daily for the duration of the study. For any dog exhibiting signs of abnormal health, the affected organ system and the specific clinical sign were recorded on the *General Health Observations Record*. Abnormal health observations recorded after administration of the initial treatment with IVP (Day 0) constituted an Adverse Event.

13.3 PHYSICAL EXAMINATION

Candidate dogs each underwent a physical examination during the acclimation period to ensure that they had no pre-existing conditions that would interfere with study objectives. Physical exams were repeated at the end of each treatment period on Days 6, 13 and 20. Physical examinations consisted of measurements of heart and respiratory rate and rectal temperature, and evaluation of the ophthalmic, otic, integumentary, neuromuscular, skeletal, respiratory, cardiac, gastrointestinal and urogenital systems. Observations were documented on the *Physical Examination Record*, and systems were evaluated as Normal or Abnormal. Any abnormal observation was characterized further by a written description in the study record.

13.4 BODY WEIGHTS

Once during acclimation and again on Days 6, 13 and 20, the body weight of each subject was measured with a verified laboratory balance that had been certified by a credentialed expert within 6 months of the onset of the study. Scales were verified with a range of test weights that incorporated one test weight lighter than the smallest dog and one test weight heavier than the largest dog, thus bracketing the anticipated weight range of all subjects. The scales were verified for accuracy before weighing the first dog and again after weighing the last dog to demonstrate the accuracy of body weights recorded on the *Body Weight and Scale Verification Record*.

13.5 POST-TREATMENT CLINICAL HEALTH OBSERVATIONS

On the first day of treatment with each dose level, (Days 0, 7 and 14), clinical assessments of health were performed prior to the A.M. treatment and at 1, 3 and 6

hours post-treatment. These observations served as a clinical assessment of systemic tolerance and evaluated behavior, vomition, salivation and any abnormalities of respiration, locomotion, feces, or other systemic signs. Observations were documented on the *Clinical Health Observations Record*. On Days 0, 7 and 14, CHOs were conducted in lieu of GHOs.

13.6 BIOLOGICAL SAMPLE COLLECTION

Blood samples were collected for complete blood counts (CBC) and clinical chemistry (CLIN CHEM) analyses on Days -4 and -1 and again on Days 3, 6, 10, 13, 17 and 20, and documented on the *Biological Sample Collection Record*.

13.6.1 CBC

Blood samples for hematologic analysis (CBC) were collected in a 3 mL evacuated tube containing K_3EDTA as an anti-coagulant. Each tube was labeled with the study number, study day or date, the donor dog's I.D. number, and the nature of the sample. Following collection, samples were mixed by gentle inversion at least five times to thoroughly mix the sample with the anti-coagulant. Samples for CBC analysis were placed into a cooler with cold packs as soon as possible after collection. Chilled samples were held under refrigeration until shipment to an analytical laboratory, on the day of collection, and transported in a cooler with cold packs.

The following hematologic tests were performed according to standard laboratory procedures:

Total white blood cell (WBC) count with differential
Absolute and proportional counts for:
- neutrophils
- lymphocytes
- monocytes
- eosinophils
- basophils

Total red blood cell count
- hemoglobin
- hematocrit (or packed cell volume, PCV)
- mean corpuscular volume
- mean corpuscular hemoglobin
- mean corpuscular hemoglobin concentration
- platelet count.

13.6.2 Clinical Chemistry

Blood samples for clinical chemistry analyses were collected in a 3 mL evacuated tube containing sodium heparin as an anti-coagulant. Each tube was labeled with the study number, study day or date, the donor dog's I.D. number, and the nature of the sample. Following collection, samples were mixed by gentle inversion at least five times to thoroughly mix the sample with the anti-coagulant. Samples for clinical chemistry analysis were placed into a cooler with wet ice as soon as possible after collection. Within one hour of collection, chemistry samples were processed by refrigerated centrifugation, and the plasma supernatant was collected with a disposable pipette and similar aliquots were transferred to two cryovials labeled as described previously. Laboratory

analysis was performed within 24 hrs of the time of collection and plasma was held under refrigeration until shipment to the analytical laboratory.

The following chemical tests were performed according to standard laboratory procedures:

Albumin
alkaline phosphatase
alanine aminotransferase
aspartate aminotransferase
urea nitrogen
calcium
chloride
cholesterol
creatinine
gamma-glutamyl transferase
globulin
glucose
phosphorus
potassium
sodium
total bilirubin
total protein
triglycerides

13.6.3 Urinalysis

Urine samples were collected once during acclimation and again on Days 6, 10, 13, 17, and 20, and documented on the *Urine Sample Collection Record*. According to testing facility SOP and standard veterinary technique, urine samples were collected by free-catch, catheterization or cystocentesis.

Each collection tube was labeled with the study number, dog ID, date and study day. As soon as possible after collection, the collection tubes were placed into a cooler containing cold packs.

Urine samples were hand-carried to a local clinical pathology laboratory for analysis. At a minimum, the following parameters were assessed: pH, specific gravity, glucose, protein, ketone bodies, bilirubin, urobilinogen, and microscopic examination of sediment (*e.g.*, crystals, casts, red blood cells, white blood cells, epithelial cells). Results were issued from the laboratory and are part of the study file.

13.6.4 Diagnostic Laboratory

Samples for CBC and Clinical Chemistry analyses were hand-carried to:

Diagnostic Laboratory Services
University of Tennessee College of Veterinary Medicine
2407 River Drive
Knoxville, TN 37996

13.6.5 Interpretation

The results of all laboratory tests were reviewed by the Attending Veterinarian. Any out-of-range values were assessed and characterized as “Clinically Significant [CS] or Not Clinically Significant [NCS]. Written comments by the Attending Veterinarian accompanied any “CS” results.

13.7 ADVERSE EVENTS

An adverse event (AE) was any observation in animals, whether or not considered to be product-related, that was unfavorable and unintended and that occurred after any administration of the IVP. Any abnormal health observations recorded after

administration of IVP on Day 0 were considered Adverse Events. AEs were documented and the nature and severity of the reaction described on the *Adverse Event Record*.

13.8 OUTCOME VARIABLES

Comparisons among treatment groups vs. control dogs were evaluated for various outcome variables, including clinical health, CBC, urinalysis and clinical chemistry analyses, body weight and food consumption. Appropriate statistical analyses will be conducted by the sponsor if apparent differences among groups are observed.

14.0 INVESTIGATIONAL VETERINARY PRODUCT ADMINISTRATION

14.1 DOSE PREPARATION

Individual doses of each IVP were prepared for all dogs assigned to the respective group. Doses of 1, 2 or 4 mg were prepared by volume on the basis of the final concentration stated on the product label.

For each individual dog, dosing personnel consulted the *Randomization and Allocation Record* to confirm its respective group assignment. A corresponding vial of IVP or saline was selected, and a calculated dose of the assigned product was aspirated into a sterile, 3 mL syringe. Each filled syringe was sealed to prevent leakage and labeled with the study number, date, and the I.D. number of the intended recipient dog. Individual doses were administered within 30 minutes of preparation. Dose preparation and administration procedures were overseen by ETCR Quality Assurance personnel and documented on the *Dose Preparation and Administration Record*.

14.2 DOSE ADMINISTRATION

Similar doses of the assigned products were administered once daily in the A.M. All doses of mitragynine or CVP were administered prior to feeding (*i.e.*, on an empty stomach).

A cooler containing prepared doses of both IVPs and control product were transported to the Small Animal Facility. Dogs were treated in the order of housing to simplify post-treatment observations. Each dog's identification number was confirmed prior to dosing.

Prior to dosing, the I.D. of the dog was confirmed and matched with the respectively labeled syringe. The dog was minimally restrained, the mouth opened gently and the contents of the syringe will be dispensed over the tongue or into the pharynx in small increments. Each dose was followed with a similar volume of tap water to assist in swallowing, if desired. Dosing activities were documented on the *Dose Preparation and Administration Record*.

All subjects were observed for vomition at ~15 minutes (±5 minutes) after each treatment.

15.0 RESULTS

15.1 DEMOGRAPHICS AND SUITABILITY FOR INCLUSION

Eighteen purpose-bred dogs were identified as candidates for the study. Sixteen met all of the inclusion and none of the exclusion criteria and were

enrolled in the study. Demographic information for the 18 candidate subjects is presented in Table 4.

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Table 4. Demographics and treatment allocations of enrolled subjects

Tattoo I.D.	Date of Birth	Breed	Gender	Group
VBI-4	5/28/24	Beagle	Female	
VYI-4	5/28/24	Beagle	Female	
AFK-4	6/7/24	Beagle	Female	
AQK-4	6/7/24	Beagle	Female	
BDK-4	6/11/24	Beagle	Female	
ZGK-4	6/8/24	Beagle	Female	
ZEK-4	6/8/24	Beagle	Female	
BOK-4	6/6/24	Beagle	Female	
BFL-4	5/7/24	Beagle	Male	
EOL-4	5/18/24	Beagle	Male	
XRL-4	6/2/24	Beagle	Male	
ALL-4	6/7/24	Beagle	Male	
ADL-4	6/7/24	Beagle	Male	
BJL-4	6/6/24	Beagle	Male	
FBL-4	6/16/24	Beagle	Male	
AAL-4	6/11/24	Beagle	Male	
xx	xx	Beagle	Female	
Xx	xx	Beagle	Male	

** dog was not selected for enrollment

15.2 PHYSICAL EXAMINATIONS

15.3 BODY WEIGHTS

Table 5. Body weights of enrolled subjects at weekly intervals

Dog I.D.	Acclimation (kg)	Day 6 (kg)	Day 13 (kg)	Day 20 (kg)	Wt. Change [1]	Group
VBI-4	9.10	9.00	8.95	8.65	(0.45)	**1**
VYI-4	8.35	8.35	8.35	8.30	(0.05)	
AFK-4	7.70	7.65	8.05	8.00	+ 0.30	**2**
AQK-4	8.30	8.30	8.20	8.35	+ 0.05	**1**
BDK-4	7.95	7.80	7.75	7.80	+ 0.15	**2**
ZGK-4	7.70	7.70	7.45	7.35	(0.35)	**1**
ZEK-4	9.30	9.25	9.40	9.20	(0.10)	**2**
BOK-4	8.55	8.55	8.55	8.65	+0.10	**xx**
BFL-4	9.80	9.80	9.65	9.75	(0.05)	
EOL-4	8.10	8.80	8.85	8.65	+ 0.55	**2**
XRL-4	9.60	9.40	9.60	9.60	0.0	**1**
ALL-4	10.20	10.25	10.05	10.35	+ 0.15	
ADL-4	8.75	8.45	8.25	8.50	(0.25)	
BJL-4	9.75	9.55	9.50	9.90	+ 0.15	
FBL-4	8.00	7.85	7.85	7.75	(0.25)	**2**
AAL-4	9.20	9.30	9.35	9.30	+ 0.10	**1**

[1] weight change (kg) over the course of the study; weight loss is recorded in parentheses and wait gain is designated by the " + " sign

Over the course of the study, the body weights of nine dogs increased or remained the same; the weights of seven dogs decreased. Weight changes ranged from a maximum loss of 0.45 kg to a maximum gain of 0.55 kg. In Group 1, xx/6 dogs gained weight, as did xx/6 dogs in Group 2. Control dogs (Group 3) xxxxx/4.

15.4 GENERAL HEALTH OBSERVATIONS

15.5 CLINICAL HEALTH OBSERVATIONS

15.6 CLINICAL PATHOLOGY

15.7 ADVERSE EVENTS

No serious Adverse Events were noted during the course of the study, but several minor health abnormalities were documented and are summarized in Table 5. The Adverse Event that were **probably** associated with treatment

was salivation. Alimentary abnormalities such as mucus or blood in the stool, vomition, and unformed feces were **possibly** associated with MGN treatment.

Table 5. Adverse Events recorded during conduct of study MGN-25-001

Dog I.D.	Date	Abnormality	Group[1]	Association with Treatment
XRL-4	30APR25	Unformed feces	**1**	Possible (1 mg)
	09MAY25	Vomit		Possible (2mg)
	19MAY25	Drooling, post-tx observation		Possible (4 mg)
AAL-4	02MAY25	Unformed feces	**1**	Possible (1 mg)
	08MAY25	Mucus in feces		Possible (2 mg)
	08MAY25	Unformed feces		Possible (2 mg)
	16MAY25	Drooling		Probable (4 mg)
	18MAY25	Drooling, pre-tx observation		Probable (4 mg)
	19MAY25	Drooling, pre-tx observation		Probable (4 mg)
	19MAY25	Excessive drooling, post-tx observation		Probable (4 mg)
BDK-4	30APR25	Blood in feces	**3**	Possible (1 mg)
	30APR25	Mucus in feces		Possible (1 mg)
	07MAY25	Blood in feces		Possible (2 mg)
	07MAY25	Mucus in feces		Possible (2 mg)
	19MAY25	Drooling, post-tx observation		Probable (4 mg)
AFK-4	30APR25	Blood in feces	**2**	Possible (1 mg)
	30APR25	Mucus in feces		Possible (1 mg)
	09MAY25	Unformed feces		Possible (2 mg)
AQK-4	05MAY25	Mucus in feces	**1**	Possible (2 mg)
	11MAY25	Blood in feces		Possible (2 mg)
	11MAY25	Mucus in feces		Possible (2 mg)
	18MAY25	Mucus in feces		Possible (4 mg)
BDK-4	07MAY25	Mucus in feces	**2**	Possible (2 mg)
VBI-4	07MAY25	Mucus in feces	**1**	Possible (2 mg)
	17MAY25	Vomit		Possible (4 mg)
ZEK-4	02MAY25	Unformed feces	**2**	Possible (1 mg)
	02MAY25	Blood in feces		Possible (1 mg)
	02MAY25	Mucus in feces		Possible (1 mg)
	08MAY25	Blood in feces		Possible (2 mg)
	17MAY25	Blood in feces		Possible (4 mg)
ZGK-4	01MAY25	Mucus in feces	1	Possible (1 mg)
	07MAY25	Blood in feces		Possible (2 mg)
	07MAY25	Mucus in feces		Possible (2 mg)

Dog I.D.	Date	Abnormality	Group[1]	Association with Treatment
	15MAY25	Mucus in feces		Possible (4 mg)
EOL-4	02MAY25	Regurgitated food	2	Possible (1 mg)
	05MAY25	Mucus in feces		Possible (1 mg)
	10MAY25	Vomit		Possible (2 mg)
	15MAY25	Regurgitated food		Possible (4 mg)
	18MAY25	Regurgitated food		Possible (4 mg)
	18MAY25	Mucus in feces		Possible (4 mg)
FBL-4	11MAY25	Unformed feces	2	Possible (2 mg)
	17MAY25	Blood in feces		Possible (4 mg)
	17MAY25	Mucus in feces		Possible (4 mg)
	17MAY25	Vomit, post-tx observation		Possible (4 mg)

[1] Group 1 = 7-hydroxy MGN; Group 2 = Pseudoindoxyl MGN; Group 3 = placebo

The frequency of Adverse Events was calculated as the total number recorded per group divided by the number of dogs in the group. The comparative frequency of AEs for the various groups were:

Group 1 (7-hydroxy mitragynine) 20/6 = 3.33

Group 2 (Pseudoindoxyl mitragynine) 19/6 = 3.16

Group 3 (placebo) 5/4 = 1.25

Thus, treatment with 7-OH MGN or Pseudoindoxyl MGN was 2.66 and 2.53 times more likely to cause Adverse Events, respectively, compared to placebo-treated controls.

Five observations of drooling in two different dogs were considered **probably** associated with treatment. It is significant to note that all episodes of drooling appeared in dogs only after the daily dose was escalated to 4 mg.

15.7.1 Adverse events at a higher dose

The study reported herein was modified from an original protocol in which escalating treatments of 10, 20 and 40 mg of MGN were to be administered twice daily. After the first dose of 10 mg, one dog (XYL-4) exhibited marked central nervous system excitation, followed by marked CNS depression. This was classified as a Serious Adverse Event and its relation to treatment with IVP was deemed “probable”, so the trial was discontinued after only one dose and the protocol was amended to reflect the study design reported herein. The amended study design features lower doses and only once daily administration.

Dog XYL-4 was normal on the day after MGN administration and was considered eligible for enrollment in the present study.

16.0 ADMINISTRATIVE ELEMENTS

16.1 REMOVAL OF SUBJECT(S) FROM THE STUDY

16.1.1 Criteria for removal of subjects from the study

A participating animal could be removed from the study if it was determined that:

- It was uncooperative with study procedures.
- It encountered a serious adverse reaction, injury, or illness dictating removal for humane reasons or necessitating treatments with concomitant medications that might confound interpretation of outcome measures.
- It died spontaneously or was euthanized.

16.1.2 Fate of removed study animals

The disposition of a participating animal removed from the study would be subject to the policies and prerogative of the test facility and would be documented on the *Early Withdrawal of Test Animals Report.*

16.2 DRUG DISPOSITION AND ACCOUNTABILITY

Quantities and identifying characteristics of the IVP delivered to the testing facility were documented on the *Investigational Veterinary Product Receipt and Accountability Record*.

16.3 ANIMAL DISPOSITION AND ACCOUNTABILITY

Individual animal records documented dog delivery, and accountability records detailed animal disposition. All animals were returned to the test facility colony unless otherwise noted in the raw data. All expired animals were disposed of according to test facility SOP and local regulations. All animal files were updated to maintain a concise history of trial participation for each individual animal. The fate of each subject, either during or after completion of the study, was documented on the *Animal Accountability and Disposition Record.*

16.4 AMENDMENTS/DEVIATIONS TO THE PROTOCOL

16.4.1 Protocol Amendments

An amendment was a written change or modification of the study protocol effected prior to implementation of the protocol or execution of the changed or modified task. Protocol Amendments included the study number, protocol title, sequential amendment number, relevant protocol sections, brief description of the amendment, and date of implementation. Required information was recorded on the *Protocol Amendment Record* and signed by the Investigator and the Sponsor Representative.

Two protocol amendments were issued for study MGN-25-001, as summarized in Table 6.

Table 6. Protocol amendments issued during conduct of the study

Amendment Number	Date Issued	Description of Amendment	Impact
1			
2			

16.4.2 Protocol Deviations

A deviation was any unapproved variation from requirements or activities stipulated in the protocol. Protocol Deviations included the study number, protocol title, sequential deviation number, relevant protocol sections, brief description of the deviation, and date of occurrence. Required information was recorded on the *Protocol Deviation Report* and signed by the Investigator and Sponsor Representative.

No protocol deviations were reported for study MGN-25-001.

16.5 COLLECTION AND RETENTION OF SOURCE DATA

All raw data were attributable, original, accurate, contemporaneous and legible. Handwritten data were written with permanent blue ink and necessary corrections were made so as not to obscure the original entry. The original raw data and the final report were sent to the sponsor for archival purposes as needed. A copy of the raw data and final report will be archived at the test facility for a period of not less than 5 yrs.

16.6 FINAL STUDY REPORT

A final report was written by the Investigator and administrative personnel and covered all elements stipulated by the protocol. Analytical results (*i.e.*, CBC, plasma chemistry and urinalysis) are included and interpreted.

17.0 ANIMAL WELFARE

The Test Facility is committed to complying with all local regulations governing the care and use of laboratory animals. Procedures were designed to avoid or minimize discomfort, distress and pain to the animals in accordance with the principles of U.S. Animal Welfare Act. In order to ensure compliance, the study protocol was reviewed and approved (ETCR-25-0333) by the Study Facility's Institutional Animal Care and Use Committee (IACUC) before the start of the trial.

18.0 CONCLUSIONS

19.0 APPENDICES

Appendix 1. IACUC Protocol Application

Appendix 2. Protocol

Appendix 3. Protocol Amendments

Appendix 4. Protocol Awareness/Trial Personnel Record; Curriculum vitae, résumés

Appendix 5. Investigational Veterinary Product(s) Receipt and Accountability Record; Shipping Records; Photo copy (test article labels)

Appendix 6. Animal Facility Information; Food label; Water analysis (monthly and annual); Facility Diagram; Certificate of Calibration (digital humidity/temp. meter); Environmental Monitoring Record

Appendix 7. Physical Examination Records; Certificate of Calibration (long stem thermometer); Laboratory Thermometer Verification

Appendix 8. Body Weight and Scale Verification Record; Scale Calibration Certificate

Appendix 9. General Health Observation Records

Appendix 10. Feeding Records

Appendix 11. Dose Preparation Training Records; Dose Calculation Record

Appendix 12. Treatment Administration Records

Appendix 13. Post-Treatment Clinical Health Observation Records

Appendix 14. Blood Sample Collection Records; Biological Sample Collection Records

Appendix 15. Plasma Processing Records; Biological Sample Processing Records; Clinical Pathology; Refrigerated Centrifuge Verification Record; Certificate of Calibration

Appendix 16. Biological Sample Collection Records (Urine); Chain of Custody

Appendix 17. Concurrent Medication Records

Appendix 18. Adverse Event Records

Appendix 19. Animal Accountability and Disposition Record

Appendix 20. Study Communications

Appendix 21. Facility SOPs