SUMMARY

This controlled laboratory study demonstrated the safety and efficacy of a test compound on the quality of life of aged dogs when compared to over the counter (OTC) anti-inflammatory products. The study consisted of 35 dogs (5 per group) randomized to one of seven treatment groups outlined in Table 1 below:

Table 1:

Group	No. of Dogs	Drug	Dose	Route	Treatment Day
1	5	Olive Oil	0.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
2	5	Test Compound (1/2X)	0.25 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
3	5	Test Compound (1X)	0.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
4	5	Test Compound (3X)	1.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
5	5	Test Compound (5X)	2.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
6	5	Cosequin®	Per label instructions	Oral	Daily
7	5	Dasuquin®	Per label instructions	Oral	Daily

Animals were individually housed and observed twice daily for mortality and morbidity during the study. Beginning during acclimation (Study Days -10 through -1) and continuing through the end of the study, daily pain assessments were conducted once daily. Physical examinations were performed once during acclimation and again at the end of the study and included body weight and body temperature. Quality of Life (QoL) Scoring was determined using a questionnaire based on the Canine Brief Pain Inventory (CBPI). Baseline QoL Data was collected once during acclimation and during weeks 2, 4 and 5 from all animals and during weeks 1 and 3 from Groups 3, 4, 6 and 7. Food consumption was measured daily beginning on Study Day -3 and body weights were measured once during acclimation and then weekly throughout the study.

1. OBJECTIVE

The objective of this study was to demonstrate the safety and efficacy of a test compound on the quality of life of aged dogs when compared to over the counter (OTC) anti-inflammatory products.

This study was conducted in accordance with VICH Guideline 9, Good Clinical Practices, Code of Federal Regulations Title 9 (Animal Welfare Act), the Study Protocol and applicable Liberty Research Inc, (LRI) Standard Operating Procedures.

2. INTRODUCTION

This study was authorized by LivePet, LLC on September 9, 2014. All procedures in this study were reviewed and approved by Liberty Research Inc. (LRI) Institutional Animal Care and Use Committee. Animals were obtained from Liberty Research, Inc., Ridglan Farms, Inc. and Covance Research Products, Inc. The in-life phase of this study began on September 26, 2014 (Study Day -10) and was completed on November 10, 2014 (Study Day 35). All records and data generated are maintained in a secured area at LRI.

3. REGULATORY COMPLIANCE

This controlled study was conducted in accordance with the following regulations and guidelines:

- VICH Guideline 9, Good Clinical Practices
- USDA Animal Welfare Regulations, Animal Welfare Act (9 CFR 1-3)
- Applicable local regulations
- Facility Standard Operating Procedures (SOPs)

4. ANIMAL CARE AND USE

All procedures performed in this study were in compliance with the USDA Animal Welfare Act (9 CFR Subchapter A), the Guide of the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996) and Liberty Research, Inc. (LRI) Standard Operating Procedures (SOPs).

5. MATERIALS AND METHODS

1.1. Study Protocol, Amendments and Deviations

The Study Protocol, Amendments, Deviations, Notes to File, Schedule of Events and Key Personnel are presented in Appendix 1. There were three Protocol Deviations prepared following study completion detailing Physical Examinations that were scheduled for Study Day 35 but were conducted on Study Day 32, missed data on Pain Inventory Questionnaires and one missed mortality check. These deviations had no impact on the outcome of the study.

1.2. Test Articles

Test Article Receipt information is presented in Appendix 2. The test compound was initially received from LivePet, LLC on 02 OCT 14 and was assigned LRI ID number T-14-0028. Four additional flasks of test compound were received on 17 OCT 14. The control article was received on

06 OCT 14 and was assigned LRI ID number T-14-0029. Cosequin® was received on 06 OCT 14 and was assigned LRI ID number T-14-0030. Dasuquin® for Dogs under 60 lbs was received on 06 OCT 14 and was assigned LRI ID number T-14-0031. Two additional bottles of Dasuquin for Dogs under 60 lbs were received on 20 OCT 14. Dasuquin® for Dogs over 60 lbs was received on 06 OCT 14 and was assigned LRI ID number T-14-0031. All test and control compounds were stored at room temperature in a secure area at the testing facility. All remaining test article was returned to the Sponsor on 25 NOV 14.

1.3. Animals

A copy of the animal procurement and disposition records are presented in Appendix 3. Fifteen hounds (female) and 1 beagle (male) were received from Covance Research Products, Inc. on 08 SEP 14, 10 beagles (female) were received from Ridglan Farms, Inc. on 16 SEP 14 and 9 beagles (8 female and 1 male) were received from the LRI colony on 19 SEP 14. These 35 animals were randomly assigned to one of seven treatment groups on Study Day -3. Randomization procedures are presented in Appendix 5. Following study completion, all animals were returned to LRI colony.

1.4. Housing and Environmental Conditions

All animals were individually housed and were separated by group. Temperatures were maintained by a central forced air gas furnace and air conditioning system. Study room temperature and relative humidity ranged from 53.0°F to 73.8°F and too low to register on the hygrometer to 74%, respectively in Iso 2 Room 1, from 53.2°F to 84.0°F and too low to register on the hygrometer to 75%, respectively in Iso 2 Room 2, from 64.2°F to 75.6°F and too low to register on the hygrometer to 63%, respectively in Iso 8 Room 1 and from 65.0°F to 77.6°F and 20% to 63%, respectively in Iso 8 Room 2. Overhead fluorescent lights on an automated 12-hour light/dark cycle were maintained throughout the study with brief interruptions for study activities. Sanitization was performed every two weeks for pens and weekly for food and water bowls.

1.5. Feed and Water Management

Diet was available to all animals *ad libitum*. Beginning on Study Day -3, food consumption was measured. The manufacturer's guaranteed nutritional analysis is included in the study file and can be found in Appendix 4.

Fresh well water was available to all animals *ad libitum* throughout the study. Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the drinking water that interferes with the results of this study. Water analyses are presented in Appendix 4.

1.6. Acclimation of Test Animals

The dogs acclimated to the study environment for ten days prior to the initial treatment. Blood samples were collected on Study Day -7, Physical Examinations and Pain Inventories were performed on Study Day -3 and body weights were collection on Study Day -1. Daily Pain Assessments were performed once daily during the exercise period and Mortality Checks were conducted twice daily throughout acclimation.

6. Inclusion Criteria

All animals used were in good overall health, had no behavioral problems and were between 4 and 8 years of age at study initiation. Examination by the Attending Veterinarian revealed health issues appropriate for the age of the animals. These findings included interdigital cysts between the toes, pressure sores, lick granulomas, soft mammary masses, eye discoloration and dental tartar.

1.8. Veterinary Care

The Staff Veterinarian was authorized to treat animals for non-study related health problems and to treat or euthanize, if appropriate. For the treatment of interdigital cysts in several animals, technicians soaked the affected feet in an Epsom Salt bath.

1.9. Randomization

Details of the randomization procedure and animal assignment to a treatment group are presented in Appendix 5. The animals were separated by breed (mongrel/beagle), then ranked by ascending Quality of Life (QoL) Scores obtained using a questionnaire based on the Canine Brief Pain Inventory (CBPI). Treatment Groups were then alternately assigned (i.e. 1, 2, 3, 4, 5, 6, 7, 1, 2, etc.).

1.10. Masking of Study

Personnel that performed observations were masked to treatment throughout the study.

1.11. Dosing Regimen and Administration

Groups 1, 2, 3, 4 and 5 received the vehicle/test compound for three consecutive days beginning on Study Day 0, then every other day for the duration of the study. Groups 6 and 7 received the over the counter anti-inflammatory medications daily beginning on Study Day 0. The table below outlines the dosing regimen and administration by group.

Group	No. of Dogs	Drug	Dose	Route	Treatment Day
1	5	Olive Oil	0.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
2	5	Test Compound (1/2X)	0.25 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
3	5	Test Compound (1X)	0.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
4	5	Test Compound (3X)	1.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
5	5	Test Compound (5X)	2.5 mg/kg	Oral	Three consecutive days beginning on Study Day 0, then every other day for the duration of the study
6	5	Cosequin®	Per label instructions	Oral	Daily

7	5	Dasuquin®	Per label instructions	Oral	Daily

Administration of the test compound was well tolerated with no indication that it was not palatable.

1.12. Sample Collection and Analysis

1.1.1. Cytokine Analysis

Blood samples were collected in serum separator tubes from all animals on Study Days -7, 0, 7, 14, 21, 28 and 35. The serum was separated and stored at H-80°C. Following study completion, serum samples from Study Days 0, 7, 14, 21, 28 and 35 were sent to Eurofins Pharma Bioanalytics Services US, Inc. for cytokine analysis.

1.1.2. Pharmacokinetic Data

Blood samples to determine pharmacokinetic parameters for the test compound were collected from all Group 3 animals prior to treatment and at 0, 0.5, 1, 2, 4, 8 hours post-treatment on Study Day 0 and 24 hours post-treatment on Study Day 1. The samples were processed, stored at H-80°C and retained to be analyzed at a later time.

1.1.3. Clinical Pathology

In order to obtain safety data, at baseline (prior to the first does) and at study completion, blood samples were collected from the animals in Groups 1, 3, 4 and 5. The following analyses were conducted:

- CBC
- Blood urea nitrogen
- Creatinine
- Glucose
- Calcium
- Sodium
- Chloride
- Potassium
- Sodium/potassium ratio
- Anion gap
- Cholesterol
- Triclyceride levels
- Phosphate
- Bicarbonate

1.1.4. Urinalysis

Urine samples were collected via catheterization or cystocentesis at baseline and at study completion. The following analyses were conducted:

- Color
- pH
- ketone
- white blood cells
- Appearance
- protein
- bilirubin
- red blood cells
- Specific gravity
- glucose
- blood
- casts

- crystals
- bacteria
- Squamous epithelia

6. STUDY OBSERVATIONS

1.1. Physical Examinations

A complete physical examination was conducted by the Attending Veterinarian on all animals once during acclimation (Study Day -3) and again at the end of the study (Study Day 32).

1.2. Mortality Checks

Mortality and morbidity checks were conducted twice daily throughout the study with the objective to assess the general health of the animals, any sign of disease and to confirm appropriate access to food and water.

1.3. Daily Pain Assessments

Animals were assessed for pain levels during their exercise period once daily throughout the study. Additional health issues were also noted during these assessments.

1.4. Quality of Life Scoring

Quality of life (QoL) Scoring was determined using a questionnaire based on the Canine Brief Pain Inventory (CBPI) as well as pain and interference with function scores. Baseline QoL data was collected form all animals once during acclimation for randomization. Additional QoL Data was collected from all groups during week 2, 4 and 5 and from Groups 3, 4, 6 and 7 during weeks 1 and 3.

1.5. Body Weights

Body Weights were obtained once during acclimation and then weekly throughout the study.

1.6. Food Consumption

Food consumption was measured daily throughout the course of the study beginning on Study Day -3.

1.7. Oxygen Saturation

Oxygen Saturation levels were obtained on Study Days -1, 7, 14, 21, 28 and 35 from Groups one, three and five through placement of a pulse oximeter.

7. DATA RETENTION

1.1. Raw Data

All records and data generated (or authenticated copies) were maintained in a secured area at the testing facility and were maintained at the study location until the study was complete. Records to be maintained included but were not limited to: animal receipts, environmental conditions, observations, storage conditions for test substance and samples; instrument identification numbers and parameters; instrument books detailing calibration checks (including annual balance calibrations) and maintenance.

1.2. Location of Raw Data and Final Study Report

The original raw data and study report will be sent to LivePet, LLC. Copies of the data and a copy of

the final study report will be maintained in the archive at Liberty Research, Inc., Waverly, New York.

8. RESULTS

1.1. Physical Examinations

Individual Physical Examination Data are presented in Appendix 6. All animals were noted to be in good general health during physical examination and any findings noted were considered appropriate for the age of the animals.

1.2. Daily Pain Assessments

Individual Daily Pain Assessment Data are presented in Appendix 7. Individual Daily Observation Data showing observations noted during Daily Pain Assessments are presented in Appendix 8. All observations were considered incidental and not related to test or control article administration.

1.3. Adverse Events

There were no Adverse Events reported during the course of the study. Observations of interdigital cysts, lick granulomas and other various skin irritations were noted in all groups prior to as well as following administration of the control article, test compound and over the counter anti-inflammatory products.

1.4. Quality of Life Scoring (QoL)

Individual Pain and Interference with Function Scores and Individual Pain Inventory Questionnaire Data and Pain are presented in Appendices 9 and 10, respectively. The biggest decrease in pain and interference with function score as well as increase in range of motion was noted in Group 3. The lowest change in the same parameters was noted in Group 1. This indicates that administration of the test compound at the therapeutic level led to a larger increase in the quality of life than the over the counter anti-inflammatory products administered to Groups 6 and 7.

1.5. Body Weights

Individual Body Weight Data are presented in Appendix 11. No notable increase or decrease in body weight changes were observed between groups over the course of the study.

1.6. Food Consumption

Individual Quantitative Feeding Data are presented in Appendix 12. No notable increase or decrease in food consumption data were observed between groups over the course of the study.

1.7. Oxygen Saturation

Individual Blood Oxygen Saturation Data are presented in Appendix 13. No notable changes in blood oxygen saturation levels were observed between groups over the course of the study.

1.8. Clinical Pathology Analysis

1.1.1. Cytokine Analysis

Changes in Group Average cytokine levels are presented in Table 1 while Individual Cytokine Analysis Data are presented in Appendix 14. The table below indicates which cytokines were analyzed.

Pro-Inflammatory	Anti-Inflammatory

GM-CSF	IL-7
IFNg	IP-10
IL-6	IL-10
IL-8	
IL-15	
MCP-1	
TNFa	
IL-2	
KC	
IL-18	

A decrease in pro-inflammatory cytokines was noted for most animals treated with the test compound following the first week of dosing. These numbers, however, increased again following the second week of dosing and continued this pattern throughout the study. This may be correlated to the when blood was collected in relation to the last dose administration. When blood samples were collected approximately 24 hours following test compound administration (Days 7, 21 and 35), a decrease in pro-inflammatory cytokines was noted indicating a decrease in inflammation. When blood samples were collected approximately 2 hours following test compound administration (Days 14 and 28), an increase in these cytokines was noted. The previous test compound administration occurred approximately 51 hours prior to this sampling. Indicating that metabolization of the test compound does not occur within 2 hours of administration and the decrease in pro-inflammatory cytokines does not last 51 hours.

When comparing the Group average from Study Day 0 to those from Study Day 35, Group 2 showed a decrease in all cytokine levels with the exception of IFNg which did not change. These decreases ranged from a 2.35% decrease in IP-10 to a 29.05% decrease in IL-2 with an average decrease of 16.28%. Groups 6 and 7 showed a decrease in 6 out of 13 cytokines analyzed with no change noted in IFNg in either group and no change in IP-10 noted in Group 6.

1.1.2. Hematology Analysis

Individual Hematology Data are presented in Appendix 15. No notable changes in hematology parameters were observed between groups over the course of the study.

1.1.3. Serum Chemistry Analysis

Individual Serum Chemistry Data are presented in Appendix 16. No notable changes in serum chemistry parameters were observed between groups over the course of the study.

1.1.4. Urinalysis

Individual Urinalysis Data are presented in Appendix 17. No notable changes in urinalysis parameters were observed between groups over the course of the study.

9. DISCUSSION & CONCLUSIONS

Dosing with the test compound was well tolerated and palatable at all dose levels (0.5X, 1X, 3X and 5X) when administered for three consecutive days and then every other day for a total of 19 dose administrations. No adverse effects related to administration of the test compound were noted in any groups. No notable changes in body weights, food consumption, blood oxygen saturation, clinical pathology or urinalysis were noted. The largest increase in Quality of life was noted in Group 3, the group receiving the test compound at the therapeutic dose level. The smallest increase in quality of life was noted in Group 1, the animals receiving the control article. A greater increase in activity level and mobility was also noted in Group 3 when compared to Groups 6 and 7, the groups receiving the over the counter anti-inflammatory products. A decrease in inflammation was indicated by a decrease in pro-inflammatory cytokines following one week of dosing in animals receiving the test compound. This decrease in inflammation was also indicated by the decrease in the group averages of 12 out of 13 cytokines in Group 2 which received the test compound at 0.5X the therapeutic dose. The 0.5X Group performed the best out of all the groups tested.

In conclusion, administration of the test compound at .5X, 1X, 3X and 5X the therapeutic dose did not indicate any toxicity, was efficacious in improving the quality of life in dogs over 3 years of age when compared to the over the counter anti-inflammatory products administered and showed a decrease in inflammation with optimal decrease in pro-inflammatory cytokines occurring in animals treated with the test compound at half the therapeutic dose when blood samples were collected approximately 24 hours following test compound administration.

Table 1

CHANGE IN GROUP AVERAGE CYTOKINE LEVELS FROM BASELINE TO STUDY DAY 35

LRI Study No. 14.5155.001
Sponsor Study No. 14-1001

Cytokine	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
GM-CSF	-7.12%	-22.41%	-2.91%	-1.08%	0.00%	1.56%	2.77%
IFNg	0.00%	0.00%	16.06%	0.00%	0.00%	0.00%	0.00%
IL-2	-2.62%	-29.06%	139.27%	9.31%	0.00%	-14.19%	9.57%
IL-6	-7.85%	-17.01%	2.48%	14.58%	67.77%	1.46%	10.97%
IL-7	-4.88%	-21.36%	-8.03%	-3.21%	-10.27%	-8.17%	-3.99%
IL-8	-13.59%	-9.03%	-5.74%	8.00%	-23.51%	-5.57%	4.81%
IL-15	-3.92%	-25.55%	1.36%	3.86%	0.00%	13.96%	-5.44%
IP-10	-4.29%	-2.35%	-2.35%	-12.77%	-54.26%	0.00%	-33.99%
KC (61)	-1.93%	-17.27%	36.72%	15.41%	18.47%	-22.54%	-23.94%
IL-10	0.72%	-7.31%	6.75%	11.36%	4.66%	-13.41%	-3.33%
IL-18	-2.91%	-28.29%	0.08%	-0.62%	31.15%	4.87%	7.29%
MCP-1	5.67%	-16.63%	-4.00%	15.61%	20.02%	-17.50%	-16.12%
TNFa	1.32%	-15.44%	-3.99%	14.11%	0.00%	0.00%	4.12%

Graph 1

GRAPH OF CHANGE IN GROUP AVERAGE CYTOKINE LEVELS FROM BASELINE TO STUDY DAY 35

LRI Study No. 14.5155.001
Sponsor Study No. 14-1001