Date of Approval: March 17, 2003

FREEDOM OF INFORMATION SUMMARY

NADA 141-217

NEUTERSOL® INJECTABLE SOLUTION for Dogs
(Zinc Gluconate Neutralized by Arginine)

Intratesticular injection for chemical sterilization in 3 to 10 month old male dogs

Sponsored by:

TECHNOLOGY TRANSFER, INC.

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FREEDOM OF INFORMATION SUMMARY

1. General information

a. File Number: NADA 141-217

b. Sponsor: Technology Transfer, Inc.
33 East Broadway, Suite 190
Columbia, Missouri 65203

Drug labeler code: 067647

c. Established Name: Zinc Gluconate Neutralized by Arginine

d. Proprietary Name: Neutersol® Injectable Solution

e. Dosage Form: Sterile, aqueous solution containing 0.2 M zinc gluconate neutralized to pH 7.0 with 0.2 M L arginine

f. How Supplied: 2 mL sterile vials

g. How Dispensed: Prescription (Rx) –Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

h. Amount of Active Ingredient: Each mL contains 13.1 mg zinc as zinc gluconate

i. Route of Administration: Intratesticular injection

j. Species/Class: Dogs

k. Recommended Dosage: One injection is administered per testicle. Dose per testicle is based on testicular width as determined by measuring each testicle at its widest point using a metric scale (mm) caliper. The dose table is illustrated in Table 1.

TABLE 1 DOSE CORRESPONDING TO TESTICULAR WIDTH

	Dose Per Testicle	
Range of Testicular Width (mm)	mL	(mg Zinc)
10-12	0.2	2.6
13-15	0.3	3.9
16-18	0.5	6.6
19-21	0.7	9.2
22-24	0.8	10.5
25-27	1.0	13.1

l. Pharmacological Category: Chemical sterilant

m. Indications: Neutersol® Injectable Solution is indicated for chemical sterilization in 3 to 10 month old male dogs.

2. Effectiveness

a. **DOSAGE CHARACTERIZATION**: Two studies were conducted to determine the dose of a single injection of zinc gluconate neutralized by arginine for chemical sterilization in dogs.

(1) Pilot Study

A 16 month pilot study was conducted to evaluate if a single intratesticular injection of zinc gluconate neutralized by arginine administered at 0.2 mL and 0.5 mL per testicle produced sterility. Twenty-seven male Beagle dogs were assigned to four groups: placebo control, surgically castrated control, and two zinc gluconate neutralized by arginine treated groups (0.2 mL and 0.5 mL). Each group contained seven dogs, four months of age except for the 0.5 mL group which contained six dogs, six months of age. The average testicular width was 11 mm in the 0.2 mL group and 18 mm in the 0.5 mL group. The dogs were evaluated to determine the treatment effect on body weight, body temperature, complete blood count, serum chemistry, testicular size (width and length), serum testosterone and semen concentration. The dogs were mated with females in heat to confirm sterility. Clinical signs and histopathology findings were evaluated. An analysis of the study results revealed the following findings in the two groups treated with zinc gluconate neutralized by arginine:

(a) The treated dogs were azoospermic (no sperm) at study termination.
(b) The treated dogs did not produce any offspring when mated with females in heat.
(c) There were no treatment related effects on body weight, body temperature, complete blood count, or blood chemistry.
(d) Five dogs exhibited adverse reactions to the injection. One dog in the 0.2 mL group developed a scrotal rupture at three months post-injection. Two other dogs in the 0.2 mL group developed draining tracts in the scrotum at one week post-injection. One dog in the 0.5 mL group developed scrotal necrosis at one week post-injection and a second dog in this group developed a scrotal erosion at four months post-injection. All wounds healed with appropriate therapy.
(e) Treated testes began to swell at 24 hours post-injection with average peak testicular size at 48 hours post-injection. By one month post-injection the average testicular size was less than on Day 0. However, there was a large amount of variability in individual dog testicle size with some dogs showing larger testicles at 1 year post-injection compared to Day 0 and other dogs with testicles that were so small they could not be measured at 1 year post-injection. There was also variability between the size of the left and right testicles on the same dog.
(f) Average serum testosterone levels at Month 15 for the placebo control (1.42 ng/mL) and treated groups (0.2 mL: 1.97 ng/mL and 0.5 mL: 1.15 ng/mL) were higher than the average of the surgically castrated dogs (0.23 ng/mL).

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Conclusions: This study demonstrated that zinc gluconate neutralized by arginine administered intratesticularly at a dose of 0.2 mL for puppies with an average testicular width of 11 mm and at a dose of 0.5 mL for puppies with an average testicular width of

18 mm was effective as a chemical sterilant. Transient testicular swelling 24-48 hours post-injection is an expected reaction to the injection. Scrotal lesions are possible and thought to be related to improper injection technique. A 25 gauge 5/8-inch needle was used in this study. To prevent possible leakage of drug onto the scrotal skin, subsequent studies used a smaller needle (28 gauge 1/2-inch).

(2) Dose Determination Study

(a) Title: Zinc Gluconate Neutralized by Arginine Administered to Dogs at 0.25, 0.30 and 0.35 mL

(b) Investigator/Study Location:

Mostafa S. Fahim, Ph.D.
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111 Allton Building, School of Medicine
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Columbia, Missouri 65212

(c) Purpose: To determine whether a single intratesticular injection of zinc gluconate neutralized by arginine causes azoospermia at the age of sexual maturity, 2) to determine the effective dose that produces sterility, and 3) to document sterility and whether or not pregnancy occurs by mating treated males with a female in heat.

(d) Animals: 40 treated male Beagle dogs, 6 months of age, with an average testicular width of 13 mm, 16.2–26 pounds body weight. 28 sexually mature, untreated, female Beagles were used for mating.

(e) Dose and Dose Groups:

The dogs were randomly assigned to four treatment groups (10 animals/group) according to testicular width (see Table 2). The right and left testicles were each measured at the widest point and the values averaged. The dogs were sedated prior to injection.

TABLE 2 TESTICULAR WIDTHS BY TREATMENT GROUP

Group	Treatment	Average Testicular Width (mm)	Range of Testicular Widths (mm)
1	Placebo Control	13.20	11.30-15.15
2	0.25 mL	13.19	11.80-15.15
3	0.30 mL	12.82	10.60-16.05
4	0.35 mL	13.13	9.95-15.35

(f) Route of Administration: Intratesticular (dorsal cranial area of each testicle at the location of the epididymis)

(g) Frequency of Treatment: One injection per testicle

(h) Duration of Study: 2 years

(i) Parameters Measured: The dogs were monitored frequently throughout the study, as described below. Semen analysis and progeny testing were considered the primary effectiveness parameters. Evaluations were extended from 12 to 24 months to demonstrate that the drug effects were not reversed with time. All male dogs were euthanized and necropsied at the end of 24 months.

When Evaluated	**Parameter**
Day -1, Day 0, monthly for 24 months	Body weight
Day -1, Day 0, 24 and 48 hours, 1 week, and monthly for 12 months	Body temperature
0.5, 1, 2 and 48 hours and 1 week	Reaction post-treatment
Day -1, Day 0, monthly for 12 months	Complete Blood Count Serum Chemistries
Day -1, Day 0, 24 and 48 hours, 1 week and monthly for 24 months	Testicular Measurements[i]
Day -1, Day 0, and Months 1, 3, 6, 9 and 12-24	Serum Testosterone Levels
Monthly from Months 4-24	Semen analysis[ii]
All male dogs were given the opportunity to mate with females in heat during the first 12 months. Two male dogs in the 0.25 mL group were given the opportunity to mate with females in heat in the second 12 month period.	Progeny testing[iii]
End of study (Month 24)	Reproductive Organ Weights Reproductive Organ Histology

[i] Testicular measurements: right and left testicular widths and lengths were averaged and measured by a caliper.

[ii] Semen analysis: sperm concentration, semen volume, and sperm motility.

Normal semen parameters [1]:	Sperm concentration	200-1000 X 10^6/ejaculate
	Semen volume	1-40 mL/ejaculate
	Spermatozoa motility	>70% with progressive forward motility

[iii] Progeny testing was initiated at 5 months post-treatment. Only two dogs were given the opportunity to mate in the second 12 month period because no other treated dogs had sperm in the ejaculate.

[1]Feldman, E, Nelson, R. Canine Male Reproduction. In: *Canine and Feline Endocrinology and Reproduction*. Philadelphia: WB Saunders Co. 1996; 682-685.

(j) Results:

1 Semen Analysis: Sperm were observed in 90-100% of the control dogs from Months 4-24. Sperm were not observed in any dogs in the treated groups at Month 4. From 6 months post-injection to the end of the study, all dogs in the 0.30 and 0.35 mL groups produced no sperm. Three dogs in the 0.25 mL group produced some sperm (oligospermia). The sperm values were too low to be considered fertile: sperm concentrations were $<2 \times 10^6$, ejaculate volumes were low (average of 0.79 mL) and sperm motilities were low (<70%). At the end of the 24 month follow-up period, 1 dog in the 0.25 mL group remained oligospermic while the other dog showed no sperm.

2 Progeny Testing: 70% of the control group males tied (intromission with ejaculation) with the females and 100% of these ties resulted in pregnancy. 20-40% of the males from the treated groups tied with the females and 0% resulted in pregnancy. The two dogs from the 0.25 mL group that were exposed to females in heat in the second 12 month period mounted the females but neither tied. One female was artificially inseminated with semen from one of these dogs, but no pregnancy resulted.

3 Reproductive Organ Weights: The mean weights of the testicles, epididymides and prostate glands of all treated groups were less than the control group.

4 Reproductive Organ Histology: Zinc gluconate neutralized by arginine administered at all doses caused severe atrophy of the testicles, epididymides and partial atrophy of the prostate glands. Histological examination revealed increased basement membrane thickness, decreased seminiferous tubule diameter, and decreased germinal epithelium heights of all treated groups compared to the control group.

5 Testicular Measurements: Mean testicular measurements of the treated groups were larger compared to the control group at 24 and 48 hours, and at 1 week post-injection. The mean testicular widths of the treated dogs decreased from Month 1 to Month 24 compared to the control group. There was a large amount of variability in individual animal testicle size with some staying the same size throughout the study and others becoming small, fibrotic and often immeasurable.

6 Serum Testosterone Levels: Mean serum testosterone levels were 41 to 52% lower in the groups treated with Neutersol® compared to the control group throughout the dose determination study. However, there were dogs in all treated groups that had testosterone levels similar to those for the control dogs at Months 1, 3, 6 and 9 and from 12 to 24 months post-injection. By Month 24, the testosterone levels for all but nine treated dogs were in the same range as the control dogs.

7 Adverse Reactions: Zinc gluconate neutralized by arginine had no effect on body weight, body temperature, complete blood counts or serum chemistries. Two dogs developed adverse reactions. One dog in the 0.3 mL group displayed mild scrotal pain from 48–72 hours post- injection. One dog in the 0.35 mL group developed an

inflamed scrotum by 48 hours post-injection which developed into a scrotal ulcer 72 hours after treatment. The ulcer was healed by week two post-injection.

(k) Conclusions: One injection of 0.25, 0.30 and 0.35 mL zinc gluconate neutralized by arginine, administered to 6 month old male Beagle dogs with an average testicular width of 13 mm produced chemical sterilization. The dose of 0.3 mL was selected as the most appropriate dose for the 13 mm testicle based on the results of the semen analyses.

Zinc gluconate neutralized by arginine caused local adverse reactions at the injection site: scrotal swelling, mild scrotal pain (1 dog) and scrotal ulceration (1 dog). The most common reaction observed was scrotal swelling and all treated testicles increased in width by 24-48 hours post injection and remained swollen until one week post treatment.

Based on the data from the pilot study and this dose determination study, a preliminary dose table was established:

TABLE 3 PRELIMINARY DOSE TABLE

Testicular Width	Dose per Testicle
11	0.2 mL
13	0.3 mL
18	0.5 mL

These data were used to determine a linear relationship between testicular width and dose and to expand the dose table to include larger and smaller testicular widths. Safety and effectiveness of the expanded dose table were confirmed in the field study using dogs with a larger range of testicular widths.

b. **SUBSTANTIAL EVIDENCE**:

(1) Title: Clinical Evaluation of a Single Intratesticular Injection of Zinc Gluconate Neutralized By Arginine (100 mg/mL) as a Chemical Sterilant in Male Puppies

(2) Investigators/Study Locations:

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(3) Purpose: To confirm the effectiveness and safety of a single intratesticular injection of zinc gluconate neutralized by arginine for chemical sterilization in 2.5-10 month old male dogs.

(4) Animals: 270 male dogs (various breeds), 2.5–10 months of age, 10-27 mm range of testicular widths, 5-78 lbs body weight. Of the 270 dogs injected, 225 completed the study. Forty-five animals failed to complete the study: failure of owners to return their animals to the clinic (28), castration (10), death (5) and euthanasia (2). Because there were not a sufficient number of 2.5 month old puppies enrolled, the drug will be labeled for use in puppies no younger than 3 months of age.

(5) Dose and Dose Groups:

Six different doses were administered according to Table 4. Doses were determined by measuring the testicles at their widest point. Testicles were measured with a SkillTech® Vernier caliper, which measured each testicle in millimeter units (mm). A one cc U-100 insulin syringe with a 28 gauge, ½ inch needle was used to inject each testicle. The skin of the scrotum was pulled tight over each testicle in order to avoid injection into the scrotum, and the needle was inserted into the dorsal cranial portion of each testicle. A slow injection technique was used to avoid leakage of the drug from the injection site. A sedative or tranquilizer was administered to 24% of the dogs (66/270) to minimize movement during injection.

TABLE 4 DOSE CORRESPONDING TO TESTICULAR WIDTH

	Dose Per Testicle	
Range of Testicular Width (mm)	mL	(mg Zinc)
10-12	0.2	2.6
13-15	0.3	3.9
16-18	0.5	6.6
19-21	0.7	9.2
22-24	0.8	10.5
25-27	1.0	13.1

(6) Control: A historical control was used. The drug's effectiveness, as determined by semen analyses, was compared to the known semen analyses of normal, healthy, intact male dogs.

(7) Route of Administration: Intratesticular (dorsal cranial portion of each testicle at the location of the epididymis)

(8) Frequency of Treatment: One injection per testicle

(9) Duration of Study: Dogs were kept at the veterinary facility for 3 days post-injection and then released and followed for at least 6 months post-injection. If adverse reactions were seen during the first 3 days post-injection, the dog remained at the veterinary facility for 7 days.

(10) Inclusion Criteria: The following criteria were utilized to qualify dogs for enrollment:

Signed owner consent form, current (up-to-date) immunizations, health status normal upon physical exam, testicular width of 10-27 mm per testicle, no cuts or ulcerations on scrotal areas or fibrosis of the testicles or epididymides and 2½ to 10-month old males with both testicles descended.

(11) Parameters Measured:

When Evaluated		**Parameter**
Day –1	Pretreatment	Physical Exam, Testicular Width
Day 0	Injection Day	Physical Exam, Testicular Width, Complete Blood Count
Day 0	0.5, 1 & 2 hours post-injection	Reaction to Injection
Days 1-3 or Days 1-7	Post-Injection Follow-up	Attitude, Appetite, Ability to Walk, Body Temperature, Scrotal Pain, Scrotal Evaluation (palpate for pain and observe for swelling, irritation, and dermatitis)
Days 3 or 7	Release from Veterinary Facility	Complete Blood Count, Physical Exam, Testicular Width
Month 6	End of Study	Physical Exam, Testicular Width and Semen Analysis

(12) Results:

(a) Effectiveness

Treatment Success: Success was defined as an animal that displayed aspermia (no semen ejaculated), azoospermia (no spermatozoa in the ejaculate), necrospermia (spermatozoa in the ejaculate are dead or motionless) or oligospermia (sperm concentration less than 20 million spermatozoa per mL) 6 months post-injection.

Treatment Failure: Failure was defined as 80% progressive forward motility and sperm concentration greater than 20 million spermatozoa per mL.

Semen was collected from 224 dogs at Month 6. Based on the criteria above, the effectiveness was 99.6%. Of the 224 dogs, there was one treatment failure, 171 dogs were aspermic (76.3%), 50 were azoospermic (22.3%), 1 was necrospermic (0.5%), and 1 was oligospermic (0.5%). The one failure is based on the dog's 6-month semen analysis: 100% motility and a sperm concentration of 165 million.

Two dogs that were treatment successes at Month 6 showed an increase in sperm concentration and motility when evaluated at Month 12. One had a sperm concentration of 49 million and 80% motility and the other had a concentration of 19 million and 100% motility.

(b) Safety: Refer to Table 6 for a summary of all adverse reactions.

Zinc gluconate neutralized by arginine had no effect on body weight or body temperature.

1 Testicular Width Measurements: Average testicular width was increased in all of the dose groups on Day 3. By Month 6, the average testicular width in all dose groups except the 0.2 mL group was less than the widths measured on Day 0. Refer to Table 5.

TABLE 5 AVERAGE TESTICULAR WIDTH (mm)

	Day 0	Day 3	Month 2	Month 6
Group				
0.2 mL	10.8	13.8	13.8	12.9
0.3 mL	14.0	17.2	15.7	13.7
0.5 mL	16.9	21.2	17.4	15.6
0.7 mL	20.1	25.0	17.7	15.7
0.8 mL	22.7	27.0	21.0	18.5
1.0 mL	26.1	29.5	23.1	17.8

2 Injection Reactions:

Reaction to the Injection: Seven dogs experienced pain on injection. The needle abraded the right side of the scrotum as one dog kicked upon drug administration. Six animals vocalized on injection.

Reaction after Injection: There were no post-injection reactions reported at 30 minutes, 1 hour or 2 hours post injection. Two dogs were reported biting and licking the scrotum on Days 3 and 5.

Attitude, Appetite, Ability to Walk: Fourteen dogs displayed abnormal appetite and attitude on Days 1, 2, 3 and 5 (attitude: 4, appetite: 9, and attitude and appetite: 1). Dogs did not display problems walking.

Scrotal Pain: Seventeen animals displayed scrotal pain post-injection on Days 1, 2, 3, and 7. The majority of these dogs displayed the pain on Days 1 and 2 post-injection when the testicles were palpated.

3 Scrotal Evaluations:

Irritation and Dermatitis: Three dogs displayed scrotal irritation on Days 1-7. Two dogs displayed irritation and dermatitis on Days 1-2.

Scrotal Reactions: One dog was reported with a perforated scrotum and a severe infection on Day 17. This dog had licked and chewed through the scrotum down to the testicle. Surgical castration and scrotal ablation were performed on Day 17. Another dog was returned to the clinic on Day 3 for an ulcerated scrotum. This dog chewed a hole through the scrotum, which became ulcerated. The dog was treated with antibiotics and sent home with an Elizabethan collar. The ulcer was healed by Day 7.

Two dogs were reported with scrotal swelling on Day 7; one dog was reported with an excessively dry scrotum on Day 7; one dog displayed scrotal bruising on Days 2, 3, and 4; one dog displayed marked preputial swelling on Day 3; one dog was reported with a scrotal sore on Day 7.

4 Vomiting: Vomiting occurred on the day of injection for 10 dogs. Timing varied from 1 minute to 4 hours post-injection. Of the 10 dogs, one dog vomited 4 times, one dog vomited 3 times, four dogs vomited 2 times and four dogs vomited once, one of which also displayed anorexia and lethargy and was recumbent on injection day. All cases resolved on their own without the use of anti-emetics.

5 Euthanasia: Two dogs were euthanized for behavioral problems.

6 Castration: One dog with a severe scrotal infection was castrated at the discretion of the veterinarian and nine dogs were castrated based on owner complaints of behavior problems, such as aggression, object mounting, and uncooperative behavior.

7 Behavioral: Nine dogs displayed male sexual behavior. The reports included aggression, leg lifting, and breeding behavior.

8 Death: One dog was found dead in the owner's yard prior to the six-month follow-up exam. The dog's body had been disposed of before a necropsy was conducted. Four dogs died from trauma

TABLE 6 ADVERSE REACTIONS

Adverse Reaction	Number of Animals (n = 270)	Percentage (%)
Reaction upon Injection		
Vocalization	6	2.2%
Kicking	1	0.3%
Local Reactions		
Scrotal Pain	17	6.3%
Scrotal Irritation	3	1.1%
Biting and Licking	2	0.7%
Scrotal Swelling	2	0.7%
Scrotal Irritation & Dermatitis	2	0.7%
Scrotal Ulceration	1	0.3%
Scrotal Infection	1	0.3%
Dry Scrotal Skin	1	0.3%
Scrotal Bruising	1	0.3%
Preputial Swelling	1	0.3%
Scrotal Sore	1	0.3%
General Reactions		
Neutrophilia	17	6.3%
Vomiting	12	4.4%
Anorexia	11	4.0%
Lethargy	6	2.2%
Diarrhea	5	1.9%
Leukocytosis	2	0.7%

(13) Conclusions: Zinc gluconate neutralized by arginine produced chemical sterilization when administered to 3-10 month old male dogs with testicular widths ranging from 10-27 mm.

Zinc gluconate neutralized by arginine caused both local adverse reactions at the injection site and systemic reactions. Transient testicular swelling occurred in all of the treated dogs by Day 3 post-injection. The injection appeared painful in 2% of the dogs and 6% of the dogs showed signs of scrotal pain after injection (primarily on Days 1-2 post-injection). Vomiting, anorexia, lethargy and neutrophilia were the most common systemic reactions seen post-injection. Signs of local scrotal irritation and bruising were seen rarely. Two dogs developed serious scrotal ulcerations and infection post-injection, necessitating castration for one of the dogs. Both cases were associated with the dog biting or licking the scrotum after release to the owner. The results of this study demonstrate that dogs should be monitored closely after injection. If there are signs of scrotal ulceration or infection, the veterinarian should be contacted immediately. Veterinarians and owners should ensure that dogs do not lick or bite the scrotal area for at least 7 days after injection.

3. Target Animal Safety

a. Title: Zinc Gluconate Neutralized by Arginine Administered at 1X, 1.5X, and 2X to Six Month Old Dogs

b. Investigator/Study Location:

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Center of Reproductive Science and Technology
111 Allton Building, School of Medicine
University of Missouri-Columbia
Columbia, Missouri 65212

c. Type of Study: GLP laboratory safety study

d. Purpose: To determine the effect of zinc gluconate neutralized by arginine administered at 1X, 1.5X and 2X the recommended dose for three times the duration of treatment.

e. Animals: 24 male Beagle dogs, 6 months of age, average testicular width of 15.2 mm, 16-21 lbs body weight.

f. Dose and Dose Groups:

The dogs were randomly placed into four treatment groups (6 animals/group) according to the pre-study testicular widths (range of 12.25-19.10 mm). The four groups included the placebo control group (Group 1: Bacteriostatic water) and the 1X (Group 2), 1.5X (Group 3), and 2X (Group 4) treated groups. Measurements of the right and left testicles were averaged to determine the dose for each dog. The 1X, 1.5X, and 2X doses are outlined in Table 7. The doses used reflect an earlier version of the proposed dosing table. The slight change in dose does not affect the conclusions drawn from the study. Acepromazine was administered to the dogs prior to the injection.

TABLE 7 DOSES USED IN THE SAFETY STUDY

	Dose Administered (mL)		
Range of Testicular Widths	1X	1.5X	2X
13-14	0.30	0.45	0.60
15-17	0.40	0.60	0.80
18-20	0.50	0.75	1.00

g. Route of Administration: Intratesticular (dorsal cranial portion of each testicle at the location of the epididymis)

h. Frequency of Treatment: One injection per testicle on Days 0, 14, and 28

i. Duration of Study: 2 months

j. Parameters Measured:

When Evaluated	**Parameter**
30 minutes, 1 hour, 2 hours	Biting or licking of testicles
30 minutes, 1, 2, 24 and 48 hours and weekly for 8 weeks	Ability to walk

Day 0, 24 and 48 hours and weekly for 8 weeks	Pain assessments Adverse reactions
Day -1, Day 0, weekly for 8 weeks	Body weight
Day -1, Day 0, 24 and 48 hours weekly for 8 weeks	Rectal temperature
Day -1, Day 0, 24 and 48 hours, weekly for 8 weeks	Testicular width and length
Day -1, Day 0, 2, 4, 6 and 8 weeks	Testosterone levels
Day -1, Day 0 and 2, 4, 6, and 8 weeks	Complete blood count
Day -1, Day 0, and 2, 4, 6, and 8 weeks	Serum chemistries (albumin, total protein, glucose, Na, K, Ca, P, ALP, SGPT, BUN, albumin/globulin ratio, creatinine)
Week 8	Necropsy (Gross) Histopathology and organ weights (testicles, epididymides, prostate gland)

k. Results:

All dogs received one injection on Day 0 and a second injection on Day 14. The third injection was attempted on Day 28 but was not administered because the testicles were too hard for the needle to penetrate and the decreased testicular size made injection difficult.

Clinical observations:

There were no reports of biting or licking at the scrotal area, leakage of the drug from the scrotum, vomiting or anorexia.

First Injection: The test material was easily injected in all dogs in the control and 1X groups. There was mild resistance to the injection (back pressure) in one dog in the 1.5 X group and severe resistance in two other dogs from this group. There was mild resistance to the injection in 5 dogs in the 2X group.

There were no adverse reactions reported in the control group. Dogs from all zinc gluconate neutralized by arginine-treated groups displayed a mild degree of discomfort (restlessness and shifting from one hip to the other) when sitting down. One dog in the 2X group displayed slight difficulty in walking 24 hours after the first injection. Three dogs in the 1.5X group displayed local reactions at the site of injection. One dog displayed a large reddened area on the scrotum 24 hours post injection. The lesion healed after 5 days of topical treatment. One dog displayed an irritated scrotum 24 hours post injection and on Day 8, one-half of the scrotum was necrotic and the testicle was

exposed. The testicle was surgically removed. There had been resistance to this injection and the dog had moved during the injection procedure. The third dog developed superficial skin necrosis on the scrotum on Day 7 and the lesion did not heal until 4 weeks after the second injection. This dog had pre-existing scrotal skin irritation prior to the injection.

Second Injection: The test material was easily injected in all control dogs and 5 dogs in each of the 1X, 1.5X and 2X groups. Resistance was felt during injection in a 1X dog. The dog moved during the injection resulting in some drug being injected into the scrotal tissue. There was resistance to the injection in the dog from the 1.5X group that still had the scrotal necrosis present from the first injection. This dog vocalized during the injection.

There were no adverse reactions reported in the control group. Five dogs in the 2X group displayed signs of pain in both testicles when examined 24 hours following the second injection. The dog in the 1X group that moved during the injection developed scrotal necrosis 48 hours post injection. The lesion healed after 5 days of topical treatment. The dog in the 2X group for which there was resistance to the injection displayed moist dermatitis of the scrotum with serous and purulent discharge starting 4 days post-injection. The lesion healed after 7 days of antibiotic treatment. A second dog in the 2X group developed scrotal dermatitis that healed after 3 days of topical treatment.

Objective data:

Zinc gluconate neutralized by arginine had no effect on body weight, body temperature, complete blood counts, serum chemistries, and gross pathology.

Testosterone Level: The average serum testosterone level of the control dogs increased over the course of the study. The average level of the 1X group increased slightly during the study and the average levels of the 1.5X and 2X groups decreased during the study. The average testosterone levels for all groups treated with zinc gluconate neutralized by arginine were less than that of the control group at week 8.

Testicular width and length: All treated dogs displayed transient testicular swelling. The peak swelling occurred between 24-48 hours after the first injection and 24 hours after the second injection.

Organ weights and histopathology: Testicular and epididymal weights were decreased in all of the treated groups compared to the control group. The prostate glands were atrophied in one dog in the 1X group, two dogs in the 1.5X group and one dog in the 2X group.

Seminiferous tubules of the treated groups displayed a greater percentage of atrophy compared to the control group (1.25% in the control group, 65.6% in the 1X group, 74.2% in the 1.5X group and 80.3% in the 2X group).

Conclusions: Zinc gluconate neutralized by arginine injected at 1X, 1.5X, and 2X the recommended dose (volume) caused the following adverse reactions: transient discomfort

when sitting down (all groups), swelling at 24-48 hours after the first and second injections (all groups), difficulty walking 24 hours after the first injection (2X), and scrotal pain at 24 hours after the second injection (2X).

Two dogs developed mild scrotal irritation. More serious scrotal lesions (purulent discharge and necrosis) were seen in 4 dogs, including 1 dog that required surgical castration due to necrosis of approximately one-half of the scrotal length. These lesions occurred in 2 dogs that moved during the injection procedure, 1 dog with a pre-existing skin lesion and 1 dog for which the injection was administered in the face of resistance. If the product contacts the scrotal skin, necrosis and ulceration are possible sequelae. Proper injection technique is critical for safe use of this product. Housing conditions (wet, cement flooring) were also considered a contributing factor in the development of the scrotal lesions. The results of this study demonstrate that the following precautions should be taken when using this product:

(1) Do not use excessive injection pressure to force the drug into the testicle. If resistance is felt, discontinue the injection immediately. Do not attempt to inject again.
(2) Do not use in dogs with pre-existing lesions on the scrotal skin.
(3) Do not shave or clip the scrotal hair or use irritating disinfectants prior to injection.
(4) Do not allow the dog to move during the injection. Chemical restraint is recommended.
(5) Do not house the dogs on hard, wet surfaces post-injection.

4. Human Safety

This drug is intended for use in dogs, which are non-food animals. Because this new animal drug is not intended for use in food-producing animals, data on human safety pertaining to drug residues in food were not required for approval of this NADA.

Human warnings are provided on the product label as follows: “Keep this and all drugs out of the reach of children. Not for human use. Wash the skin with soap and water and flush eyes with copious amounts of water if contact occurs. Flush mouth with water and drink plenty of water if accidental ingestion occurs. Contact a physician if accidental exposure occurs by any route (dermal, oral or injection).”

5. Agency Conclusions

The data submitted in support of this NADA satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and Part 514 of the implementing regulations. The data demonstrate that Neutersol® (zinc gluconate neutralized by arginine) Injectable Solution for 3-10 month old male dogs, when used under labeled conditions of use, is safe and effective for chemical sterilization.

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is required to properly administer the injection, prescribe chemical restraint if needed, provide adequate instructions for post treatment care, and to monitor the safe use of the product, including treatment of any adverse reactions.

Under section 512(c)(2)(F)(i) of the FFDCA, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has previously been approved.

Technology Transfer holds the following patents.

Patent No.	**Expiration**
4,937,234	08/10/08
5,070,080	01/30/09

6. Labeling (attached)

Vial Label
Carton Label
Package Insert
Client Information Sheet