Date of Approval: July 25, 2011

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-325

INCURIN
(Estriol)
Tablets
Dogs

For the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs.

Sponsored by:

Intervet, Inc.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-325
B. Sponsor:	Intervet Inc. 556 Morris Avenue Summit, NJ 07901 Drug Labeler Code: 000061
C. Proprietary Name(s):	INCURIN
D. Established Name(s):	Estriol
E. Pharmacological Category:	Hormone
F. Dosage Form(s):	Single-scored tablet
G. Amount of Active Ingredient(s):	1 mg of estriol per tablet
H. How Supplied:	INCURIN Tablets are supplied in foil-sealed blister packs of 30 single-scored tablets. Two carton sizes containing either 30 tablets (one blister pack) or 90 tablets (three blister packs) are available.
I. How Dispensed:	Rx
J. Dosage(s):	The dose of INCURIN Tablets is not dependent upon body weight. All dogs should receive an initial dose of 2 mg INCURIN Tablets (2 tablets) orally once per day for a minimum of 14 days. After urinary incontinence is controlled, the lowest effective daily dose of INCURIN Tablets should be determined by decreasing the dose in a step-wise manner from 2 mg once daily (2 tablets) to 1 mg once daily (1 tablet), then 0.5 mg once daily (1/2 tablet) depending upon the response of the individual dog. There should be a minimum of 7 days between each dose adjustment. After the lowest daily dose that controls urinary incontinence is identified, the dose may be decreased further by administering once every two days. Dogs should not receive more than 2 mg INCURIN Tablets per day (2 tablets). If the dog does not respond to 2 mg of INCURIN Tablets per day, the diagnosis should be re-assessed.

K. Route(s) of Administration: Oral

L. Species/Class(es): Dog, ovariohysterectomized females

M. Indication(s): For the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs.

II. EFFECTIVENESS:

A. Dosage Characterization:

The dose was characterized by conducting a pharmacokinetic study and a field study.

1. Pharmacokinetic Study

Study Title: Plasma pharmacokinetics of estriol in dogs after oral administration of estriol tablets.

Type of Study: Multiple dose pharmacokinetic study

Investigator:
Dr. B.P.M. Janszen
Intervet International B.V.
Boxmeer, The Netherlands

Purpose: To measure the plasma pharmacokinetics of estriol tablets when given orally at the intended maximum daily dosage of 2 mg per dog per day for 7 days.

Animals: Six healthy female Beagle dogs, ranging in age from 1 to 2 years and in body weight from 10 to 12 kg

Dosage Form: Tablets containing 1 mg of estriol per tablet

Route of Administration: Oral

Dosage: Two mg of estriol per dog per day administered 15-30 minutes before the daily food ration

Test Duration: Seven consecutive days of treatment

Variables Measured: The concentration of estriol in plasma was determined on Days 1, 3 and 7 at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dosing.

Data Analysis: Plasma pharmacokinetic variables were calculated according to non-compartmental analysis.

Results: Plasma concentrations and pharmacokinetic variables of estriol varied among dogs. The mean pharmacokinetic variables (WinNonlin 4.0) of estriol in female dogs (n=6) following once daily oral administration (2 mg/dog) for 7 days are summarized in Table 1.

Table 1: Pharmacokinetic Results

ID	Dosing Day	$T_{1/2}$ Lambda Z	T_{max}	C_{max}	C_{last} (T_{last} = 24)	AUC_{0-24}
		hr	hr	pg/mL	pg/mL	hr*pg/mL
Mean	1	9.1	0.8	618	44	2075
SD	1	3.6	0.3	342	26	787
Mean	3	12.1	0.7	595	55	2865
SD	3	7.3	0.3	275	34	740
Mean	7	7.8	0.8	740	36	3639
SD	7	3.6	0.6	258	30	1495

Rapid absorption (T_{max}<1 hr) was observed in dogs. Plasma estriol concentrations returned to pre-treatment values within 48 hours of administration of the last dose. A second peak plasma concentration occurred around 4-12 hrs post-dosing in most (5/6) of the dogs tested, indicating the potential existence of entero-hepatic recirculation and drug re-absorption. Since AUC_{0-24} increased with repeated dosing, increased systemic exposure should be expected with repeated dosing of estriol in dogs.

Adverse Events: No adverse events were observed during this study.

Conclusions: This study confirms the maximum intended dose rate of 2 mg of estriol per day. The study shows that increased systemic exposure occurred with repeated dosing. These results are also consistent with the target animal safety study and clinical trials, including long-term treatment, which confirm that no signs of bone marrow suppression (or estriol accumulation) were observed in dogs receiving estriol. This is probably due to the short-acting characteristic of this natural estrogen.

2. Field Study

Study Title: A field effectiveness and safety study with INCURIN Tablets in dogs.

Type of Study: Dose characterization study

Investigator:
Dr. T. Nell
Intervet International B.V.
Boxmeer, The Netherlands

Purpose: To assess the effectiveness and safety of estriol for the control of urinary incontinence in dogs under field conditions in Europe using a self-

controlled study design.

Animals: One hundred thirty-six female dogs (133 ovariohysterectomized, 3 intact) of at least 1 year of age were enrolled in the trial.

Dosage Form: Scored tablets containing 1 mg of estriol per tablet

Route of Administration: Oral

Dosage: The initial dose rate was 2 mg of estriol (two 1 mg tablets) per dog per day for 7 days. If the dog responded (continent or improved) the dose was reduced to 1 mg daily for 7 days and then to 0.5 mg daily for 7 days. Once the dog was receiving the lowest effective daily dose of estriol for at least 1 week, alternate day treatment using the same dose was attempted. If incontinence recurred, the dose was returned to and maintained at the lowest effective daily dose.

Test Duration: Each dog was evaluated over a 42 day period.

Variables Measured: The primary variable was the reduction of uncontrolled urine loss as evaluated by the Investigator and owner on Days 7, 28 and 42. Dogs were classified as Responders (no incontinence or improved) or Non-Responders (unchanged or worse). Other signs of incontinence (wetness, urine smell) were also evaluated. Blood samples for complete blood counts were obtained prior to treatment (Day 0) and at the end of the study (Day 42). Mammary glands were examined at the beginning and end of the study.

Results: Table 2 shows the results of the continence observations at each time point. There was no relationship between the severity of initial clinical signs and the response to treatment. There was no relationship between body weight and the final dose or dose regimen. Of those dogs classified as responders at Day 42 (no incontinence or improved), 70% were on a once daily administration schedule. The complete blood counts revealed no abnormalities.

Table 2: Continence Responses

Response	Day of Observation		
	Day 7	**Day 28**	**Day 42**
No incontinence	64 (47.1%)	74 (59.7%)	79 (66.9%)
Improved	54 (39.7%)	28 (22.3%)	27 (22.9%)
Unchanged (but continued on study)	5 (3.7%)	7 (5.6%)	4 (3.4%)
Worsened (but continued on study)	0 (0%)	5 (4.0%)	3 (2.5%)
Treatment failure	9 (6.7%)	5 (4.0%)	3 (2.5%)
Missing responses	1 (0.7%)	4 (3.2%)	2 (1.7%)
Removed/died[1]	3 (2.2%)	1 (0.8%)	0 (0%)
Total	136	124[2]	118[3]

[1]These dogs were removed from the study due to circumstances unrelated to the drug, hence they are not considered treatment failures.
[2]The 12 dogs (9 treatment failures and 3 removals/deaths) from the day 7 time point did not generate any data at the day 28 time point. Therefore, only 124 cases remain. The percentages were calculated using 124 as the denominator.
[3]The 6 dogs (5 treatment failures and one removal/death) from the day 28 time point, in addition to the 12 dogs from the day 7 time point, did not generate any data. Therefore, only 118 cases remain at the day 42 time point. The percentages were calculated using 118 as the denominator.

Adverse Reactions: The following adverse reactions were observed and are listed in decreasing order of frequency: estrogenic (swollen, reddened, bleeding or licking of vulva, and swollen or licking of mammary glands) and gastrointestinal (inappetence and vomiting) effects. These effects were most often noted at the beginning of drug administration when higher doses of estriol were used. These effects usually improved after the dose was decreased.

Conclusions: At day 42, 89.8% of the 118 evaluable dogs in the study responded favorably (no incontinence or improved) to the estriol administration. A starting dose of 2.0 mg estriol administered orally once daily with dose reduction based on clinical response resulted in a final dose between 0.5 to 2.0 mg daily or every other day. Of those dogs that responded favorably, 70% required a once daily maintenance dose. However, the final dose and dose regimen should be established for each individual dog. The hematology results did not show any evidence of bone marrow suppression due to the estriol administration. The most common adverse reactions were estrogenic and gastrointestinal, but these signs generally occurred at the highest dose and improved or resolved once the dose was reduced.

B. Substantial Evidence:

Study Title and Number: A placebo-controlled, field clinical effectiveness study of estriol (INCURIN Tablets) for the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs (Study No. 2024-006-00).

Type of Study: Multi-site field safety and clinical effectiveness study in client-owned dogs.

Study Dates: January 30, 2007 to November 4, 2008 (in-life phase).

Locations and Investigators:

Dr. Troy Smith Austin, TX	Dr. Mark Lapierre Greensboro, NC	Dr. Nancy Peterson Des Moines, IA
Dr. Jay Butan Lake Worth, FL	Dr. Corey Entriken Gladstone, MO	Dr. Courtney Rebensdorf Cranston, RI
Dr. Beth Carroll Durham, NC	Dr. Robert Thompson Bear, DE	Dr. Linda Hanson Cumberland, RI
Dr. Terry Clekis Bradenton, FL	Dr. William Urban Sunbury, OH	Dr. Susan Hubbard Rochester, NY
Dr. Donald Dinges Leawood, KS	Dr. Roberta Jackson Dover, DE	Dr. Peter Davis Augusta, ME
Dr. Sam Geller Quakertown, PA	Dr. Donna Namey Wilmington, DE	Dr. Stephanie DeMarco Newark, DE
Dr. Stuart Gluckman Mendon, NY	Dr. John Teeter Shawnee Mission, KS	Dr. Steve Hodes Mine Hill, NJ
Dr. Wally Diehl Chapel Hill, NC	Dr. Paul Black Rochester, NY	Dr. Lilli Kusiak Newtown, PA
Dr. Stephanie DeMarco Wilmington, DE	Dr. Jason Smith Chapel Hill, NC	Dr. Max Heimlich Spring, TX

Study Design: The study had 2 phases, an effectiveness phase and a dose titration phase. The first phase determined effectiveness of the drug in estriol-treated versus placebo-treated dogs (Day 0 to Day 14). During the second phase, all enrolled dogs were treated with estriol (Day 14 to Day 42), and the dose was decreased weekly, if possible, to identify the lowest dose of estriol needed to control urinary incontinence in individual dogs.

Objective: To evaluate the effectiveness of estriol tablets for the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs.

Description of Test Animals: Two hundred and twenty six (226) client-owned, ovariohysterectomized female dogs 1 year of age or older with a minimum of 3 urinary incontinence episodes per week were enrolled at 27 sites. Two-hundred and six dogs (106 estriol-treated, 100 placebo-treated) from 22 sites were included in the effectiveness analysis. Dogs ranged in age from 1 to 17 years (mean age 7.3 years in estriol group and 7.9 years in placebo group). A total of 60 breeds were represented, the most common breed being mixed breed, followed by Labrador retriever, Doberman, Boxer, and German shepherd. The mean body weight of dogs in the estriol-treated group (28.7 kg) was similar to that of the placebo-treated group (27.3 kg). All 226 dogs (115 estriol-treated and 111 placebo-treated) were included in the safety analysis.

Treatment Groups (Phase 1): There were 2 treatment groups in the Effectiveness phase, as shown in Table 1.

Table 1: Description of Treatment Groups and Doses for Effectiveness Phase

Tx Group	**Dose**	**Number and Gender of Animals**
Estriol	2 tablets (1 mg each) once daily for 14 days	106 ovariohysterectomized female dogs
Placebo	2 tablets once daily for 14 days	100 ovariohysterectomized female dogs

Randomization: Dogs qualifying for enrollment were blocked based on site, paired based on order of entry within a site, and randomized to treatment with either estriol or placebo.

Masking: All study site personnel participating in effectiveness evaluations and all dog owners were masked to treatment assignment during the Effectiveness phase (Day 0 to Day 14). Randomization and allocation documents were prepared in advance and supplied to each study site. Treatment coordinators not masked to treatment used the prepared documents to assign dogs to treatment groups and to dispense the assigned treatment. During the Titration phase (Day 14 to Day 42), all enrolled dogs were treated with estriol and the study was unmasked.

Inclusion Criteria:

- Female ovariohysterectomized dogs 1 year of age or older
- History and clinical signs of urinary incontinence (≥ 3 episodes per week as documented in a pre-enrollment incontinence diary kept by the owner)
- Deemed eligible by the Investigator based on physical exam and clinical pathology results
- Signed statement of informed consent by owner

Exclusion Criteria: Dogs exhibiting 1 or more of the following were not eligible for enrollment:

- A severe life threatening medical condition(s)
- Evidence on physical examination, serum chemistry, or hematology of concurrent illness that could affect evaluation of treatment
- A serious disorder(s) requiring regular systemic treatment, unless the dog was in a stabilized clinical condition
- Evidence of an active urinary tract infection (dogs with a documented urinary tract infection within the 2 weeks prior to enrollment were required to be free of signs of urinary infection and have a negative urine culture before being enrolled in the study)
- Polydipsia and/or polyuria
- Receiving contraindicated therapy

Drug Administration:

Effectiveness phase (Phase 1): Estriol-treated dogs received a dose of 2 mg (two 1 mg tablets) orally once per day for 14 days. Control dogs received 2 placebo tablets orally once per day for 14 days.

Titration phase (Phase 2): Starting on Day 14, all dogs completing the Effectiveness phase began receiving 2 mg of estriol orally once per day. At each of 3 subsequent weekly assessments (Days 21, 28, and 35), the Investigator evaluated the patient response based upon the assessment interview with the owner. The Investigator was permitted to adjust the dosage up or down (not to exceed 2 mg per day) in a step-wise fashion using the following dose regimens:

Figure 1: Dose Adjustments for Titration Phase

	Dose regimen
Maximum dose	***2 mg once daily***
	1 mg once daily
	0.5 mg once daily
Minimum dose	***0.5 mg every other day***

Dose adjustment began with an optional single step decrease at the Day 21 assessment. If urinary incontinence worsened following a dose decrease, the Investigator could increase the dose a single step at the next assessment. If urinary incontinence continued to be controlled, the Investigator could decrease the dose again by a single step at the next assessment. The Titration phase lasted through Day 42.

Study Variables During the Effectiveness Phase: The study used a combination of 2 variables to evaluate effectiveness:

- Response to Treatment scored by the Investigator on Days 7 and 14
- Frequency of urinary incontinence episodes as recorded by the owner daily.

a. Response to Treatment variable: The Investigator assessed each dog's Response to Treatment on Day 7 and Day 14 of the Effectiveness phase relative to the condition of the dog on Day 0, and each dog was scored as continent (Score 1), improved (Score 2), no change (Score 3), or worse (Score 4). The Investigator scored the dog based upon the clinical assessment (physical examination, wetness of the perineum, and urine smell on the dog) and on comments and observations by the owner (number of urinary incontinence episodes as recorded in the owner diary for the previous week, incontinence signs, and the owner evaluation of the dog's condition compared to baseline). Dogs scored as continent (1) or improved (2) were classified as Responders. Dogs scored as no change (3) or worse (4) were classified as Non-Responders, as shown in Table 2. Dogs had to be classified as Responders to achieve treatment success.

Table 2: Response to Treatment Criteria

Response to Treatment	Score	Change	Definition of change
Responder	1	Continent	No urinary incontinence.
	2	Improved	Urinary incontinence reduced compared to Day 0.
Non-Responder	3	No change	Urinary incontinence was the same compared to Day 0.
	4	Worse	Urinary incontinence was worse compared to Day 0.

b. Frequency of urinary incontinence episodes: During the pre-enrollment phase (Day -7 to 0), and during the Effectiveness Phase (Day 0 to 14), dog owners recorded the daily incidence of urinary incontinence episodes in an owner diary. The Investigator used the pre-enrollment phase observations to qualify the dog for study enrollment, and to establish a baseline incontinence episode frequency per week. At the Day 7 and Day 14 visits, the Investigator recorded the number of incontinence episodes from the prior week to determine the frequency of episodes for each week. The study compared the incontinence episode frequency from the second week to the baseline episode frequency to determine whether the dog achieved a targeted episode reduction necessary for treatment success. The Investigator also used the weekly frequency of urinary incontinence episodes in conjunction with the clinical examination and owner evaluation to determine the Response to Treatment variable.

Treatment Success: The primary effectiveness outcome variable in Phase 1 of the study was Treatment Success at Day 14, which incorporated the Response to Treatment variable and a targeted reduction in the frequency of urinary incontinence episodes. A dog was considered a Treatment Success if it: a) was categorized into the Responder category for the Response to Treatment variable at Day 14 (continent, Score 1, or improved, Score 2), and b) experienced a decrease in the number of urinary incontinence episodes from Day 7 to Day 14 compared to baseline as described in Table 3 below.

Table 3: Definition of Treatment Success in Phase 1 of the Study

Response to Treatment, Day 14		**Targeted Reduction in the Frequency of Urinary Incontinence Episodes**
Responder Score = 1 (Continent) **OR** Responder Score = 2 (Improved)	**AND**	Baseline (Day -7 to Day 0) frequency was 3 episodes per week, and the dog experienced a decrease of at least 2 episodes per week from Day 7 to Day 14 **OR** Baseline (Day -7 to Day 0) frequency was 4 to 7 episodes per week, and the dog experienced a decrease of at least 3 episodes per week from Day 7 to Day 14 **OR** Baseline (Day -7 to Day 0) frequency was 8 or more episodes per week, and the dog experienced a decrease of at least 50% in the number of episodes per week from Day 7 to Day 14

Secondary Variables:

a. Frequency of urinary incontinence episodes: Recorded during the 1 week period prior to the Day 7 and Day 14 visits (from the owner diary) and evaluated independently of the Response to Treatment variable. Owners did not record the actual number of urinary incontinence episodes after Day 14.

b. Owner evaluation: The owners provided a subjective evaluation of their dogs' signs of urinary incontinence on Days 7 and 14 of the Effectiveness phase, and on Days 21, 28, 35, and 42 of the Titration phase. Investigators asked the owners to rate the dog's condition compared to Day 0 as "better", "the same", or "worse".

Other clinical variables:

a. Physical examination and body weight: Performed pre-treatment, Day 7, and Day 14 of the Effectiveness phase; and Days 28 and 42 of the Titration phase

b. Hematology and serum chemistry: Performed pre-treatment and Day 14 of the Effectiveness phase, and Day 42 of the Titration phase

c. Urinalysis and urine culture: Performed pre-treatment only

Statistical Analysis: Statistical analysis of the primary variable Day 14 Treatment Success was conducted using a generalized linear mixed model employing a binomial distribution with logit link. The model contained treatment as a fixed effect, site and site-by-treatment interaction as random effects. The analysis used the Kenward-Roger option to estimate degree of freedom and the main effect of treatment was evaluated at alpha=0.05 significance level. For the secondary variables, Day 14 frequency of urinary incontinence episodes and Day 14 owner evaluation of dog's condition were analyzed using the Cochran-Mantel-Haenszel test with site as a stratum.

Results: The proportion of Day 14 Treatment Successes in the estriol-treated group (66%) was statistically significantly greater (P = 0.0038) than in the placebo-treated group (37%) as shown in Table 4.

Table 4: Summary of Treatment Successes at Day 14

Treatment Group	**Number of Cases**	**Number of Successes**	**Percent Success**	**P-Value for Comparison of Treatment Successes**
Estriol	106	70	66%	**P = 0.0038**
Placebo	100	37	37%	

a. Response to Treatment at Day 14: Investigators classified a greater proportion of estriol-treated dogs as Responders at Day 14 than placebo-treated dogs. Table 5 shows that 78 of 106 dogs in the estriol group (73.6%) were Responders compared to only 53 of 100 dogs in the placebo group (53%). There were 8 estriol-treated and 16 placebo-treated dogs classified as Responders that did not achieve Treatment Success.

Table 5: Summary of Response to Treatment at Day 14

Treatment Group	Response Category	Score	Number and Percent of Dogs
Estriol	Responder	1 (Continent)	26 / 106 = 24.5%
		2 (Improved)	52 / 106 = 49.1%
	Non-Responder	3 (No Change)	19 / 106 = 17.9%
		4 (Worse)	9 / 106 = 8.5%
Placebo	Responder	1 (Continent)	10 / 100 = 10.0%
		2 (Improved)	43 / 100 = 43.0%
	Non-Responder	3 (No Change)	36 / 100 = 36.0%
		4 (Worse)	10 / 100 = 10.0%
		Not scored*	1 / 100 = 1.0%

*One dog in the placebo group was removed from the study on Day 6 due to diarrhea

b. Frequency of urinary incontinence episodes at Day 14: A greater proportion of estriol-treated dogs met the target incontinence episode success criteria by Day 14 than placebo-treated dogs. Table 6 shows that 74 of 106 estriol-treated dogs (69.8%) reached the targeted reduction in incontinence episodes compared to only 40 of 100 dogs placebo-treated dogs (40%). There were 4 estriol-treated and 3 placebo-treated dogs that met the target episode criteria but were not Treatment Successes.

Table 6: Summary of Frequency of Urinary Incontinence Episodes at Day 14

Treatment Group	Baseline (Day 0) Frequency of Episodes/Week	Effectiveness Target (Day 14) Episodes/Week	Number and % of dogs meeting target episodes
Estriol	3 episodes/week	Decrease of at least 2 episodes/week	4 / 5 = 80.0%
	4 - 7 episodes/week	Decrease of at least 3 episodes/week	21 / 29 = 72.4%
	8 or more episodes/week	Decrease of at least 50% in episodes/week	49 / 72 = 68.1%
	TOTAL		**74 / 106 = 69.8%**
Placebo	3 episodes/week	Decrease of at least 2 episodes/week	3 / 9 = 33.3%
	4 - 7 episodes/week	Decrease of at least 3 episodes/week	9 / 21 = 42.9%
	8 or more episodes/week	Decrease of at least 50% in episodes/week	28 / 70 = 40.0%
	TOTAL		**40 / 100 = 40.0%**

Secondary Variables:

a. Mean frequency of urinary incontinence episodes: Table 7 below shows that the estriol-treated group had a greater decrease in the mean number of urinary incontinence episodes by Day 7 than the placebo-treated group relative to Day 0. By Day 14, the mean number of episodes continued to decline only in the estriol-treated group.

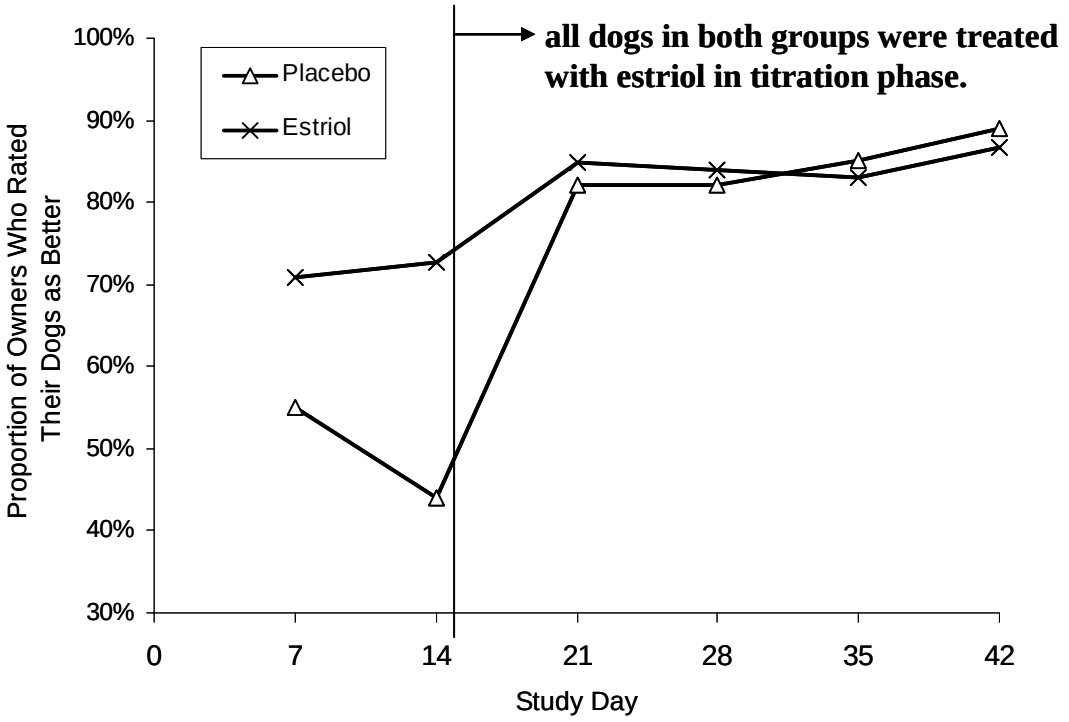
Table 7: Summary of Mean Urinary Incontinence Episodes per Week

Group	Item	Day 0	Day 7	Day 14
Estriol	# of dogs	106	105	106
	Mean # of episodes	**14.2**	**8.2**	**6.5**
	Standard deviation ±	11.2	8.1	9.8
Placebo	# of dogs	100	99	99
	Mean # of episodes	**15.8**	**11.2**	**11.3**
	Standard deviation ±	14.4	13.5	13.8

Owner evaluation: The proportion of owners who considered their dogs to be better at Day 14 compared to Day 0 was greater in the estriol-treated group (72.6%) than the placebo-treated group (44%). Once placebo-treated dogs began receiving estriol in the Titration phase, the proportion of owners whose dogs were rated as better compared to Day 0 increased considerably by Day 21, and was comparable to owners

whose dogs received estriol starting at Day 0. Figure 1 shows how the percentage of owners who considered their dogs to be better increased over the duration of the study.

Figure 2: Proportion of owners who rated their dogs' urinary incontinence as better (compared to Day 0). Placebo-treated dogs received estriol after Day 14.



Other clinical variables:

a. Physical examination: Aside from reported adverse reactions, there were no additional estriol-related physical examination findings.

b. Body weight: During the Effectiveness phase, approximately one-half of the dogs in both groups gained weight and the other half lost weight. Over the 42-day study, 56% of the dogs treated with estriol for both study phases lost weight, compared to 60% of the dogs initially treated with placebo for 14 days during the Effectiveness phase and then estriol for 28 days during the Titration phase. The majority of the dogs that experienced weight loss in both groups lost ≤ 5% of their body weight. A small percentage of dogs in both groups (3.7% in estriol/estriol group and 2% in placebo/estriol group) had marked weight loss of >10 to 20% body weight from Day 0 to Day 42.

c. Hematology and serum chemistry: Three estriol-treated dogs had white blood cell and absolute neutrophil counts that shifted above the normal range on Day 14 of the Effectiveness phase, whereas no placebo-treated

dogs did. The increases were transient and had resolved by Day 42; there were no associated clinical consequences. More dogs in the estriol-treated group (6) had hematocrit values that shifted above normal at Day 14 than the placebo-treated group (2), but these increases had no clinical impact.

Titration (Phase 2) dosing results: At the end of the study, dose information and urinary incontinence scoring was jointly available for 191 dogs. Of these dogs, 94 (49%) were receiving 2 mg of estriol daily, and 97 (51%) were receiving less than 2 mg daily, as shown in Table 8. Among the 94 dogs receiving 2 mg of estriol daily, 81 dogs (86%) had either reduced urinary incontinence compared to Day 0 (n = 57) or no urinary incontinence (n = 24). Of the ninety-seven dogs with dose reductions below 2 mg per day, all dogs receiving 1 mg of estriol once daily (n = 41), 1 mg every other day (n = 5), or 0.5 mg every other day (n = 24) had either reduced or no incontinence compared to Day 0. Twenty-five of 26 dogs receiving 0.5 mg once daily had reduced or no incontinence, and one dog dosed with 2 mg estriol every other day had reduced incontinence.

Table 8: Estriol Doses in Use during Final Week and Response to Treatment at Day 42

Dose of Estriol In Use During the Final Week of the Study	# of cases	Urinary Incontinence Description and Score			
		None score=1	Reduced Compared to Day 0 score=2	Same Compared to Day 0 score=3	Worse Compared to Day 0 score=4
		# of Responders		# of Non-Responders	
0.5 mg, Every other day	24	17	7	0	0
0.5 mg, Every day	26	17	8	1	0
1 mg, Every other day	5	3	2	0	0
1 mg, Every day	41	29	12	0	0
2 mg, Every other day	1	0	1	0	0
2 mg, Every day	94	24	57	12	1
TOTAL	191	90	87	13	1

Adverse Reactions: No dogs died during the study. Three of 224 dogs treated with estriol were removed from the study in association with possible drug-related adverse reactions; one dog developed cystitis and a second dog had lethargy and inappetence. The third dog, originally in the placebo group, received estriol for 10 days during the Titration phase and was removed from the study on Day 23 due to suspected uterine stump pyometra. The relationship between this adverse reaction and estriol could not be definitely determined.

Table 9 lists the most common adverse reactions reported during the Effectiveness phase that occurred at a higher frequency in the estriol-treated dogs compared to the placebo-treated dogs. The most common adverse reactions were gastrointestinal (anorexia, emesis) and estrogenic (swollen vulva, attractiveness).

Table 9: Summary of Adverse Reactions during Effectiveness Phase

Adverse Reaction	Estriol group % of dogs (n = 115)	Placebo group % of dogs (n = 111)
Anorexia	13.0%	3.6%
Emesis	9.6%	6.3%
Polydipsia	7.0%	7.2%
Swollen vulva	4.3%	0.0%
Anxiety	3.5%	2.7%
Sexual attractiveness	3.5%	1.8%
Somnolence	1.7%	0.0%
Hypersalivation	1.7%	0.0%
Estrous behavior	0.9%	0.0%
Mammary hyperplasia	0.9%	0.0%

Table 10 lists the adverse reactions reported during the Titration phase. The most common adverse reactions were gastrointestinal (anorexia, emesis, and diarrhea) and estrogenic (licking vulva, swollen vulva, vulvovaginitis).

Table 10: Summary of Adverse Reactions during Titration Phase

Adverse Reaction	Estriol (n = 224)
Anorexia	12.9%
Emesis	7.6%
Licking vulva	4.5%
Swollen vulva	4.0%
Vulvovaginitis	4.0%
Lethargy	4.0%
Polydipsia	3.6%
Aggression	1.8%
Cystitis	1.3%
Hyperactivity	1.8%
Mammary hyperplasia	1.3%
Anxiety	0.9%
Somnolence	0.9%
Estrous behavior	0.9%
Sexual attractiveness	0.4%
Alopecia (local)	0.4%

Conclusions: Estriol administered orally at 2 mg per dog per day for 14 days was effective for the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs 1 year of age and older. Following the initial 14-day treatment, estriol continued to control urinary incontinence at doses less than 2 mg per day in approximately 50% of dogs in the study. The most common adverse reactions associated with the administration of estriol were gastrointestinal (anorexia, emesis) and estrogenic (swollen vulva, vulvovaginitis).

III. TARGET ANIMAL SAFETY:

A. Target Animal Safety Study:

Study Title and Number: Target animal safety study in Beagle dogs administered INCURIN (estriol) Tablets orally at 1X, 3X and 5X the intended maximum labeled dose rate daily for 26 weeks (Study No. 2024-002-01).

Study Dates: January 8, 2004 to July 2, 2004 (in-life phase)

Investigator:
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Objective: To evaluate the safety of estriol administered orally to healthy dogs at doses of 0, 2, 6, and 10 mg per dog once daily (0, 1X, 3X, and 5X the maximum proposed label dose of 2 mg of estriol per dog per day) for 26 weeks.

Animals: Twenty four healthy, purebred female ovariohysterectomized Beagle dogs approximately 1 year of age weighing 7.3 to 10.5 kg at the beginning of the treatment period were allocated to 4 treatment groups of 6 dogs each.

Test Material: Single scored tablets containing 1 mg of estriol.

Dosage: 0, 2, 6, and 10 mg of estriol per dog (0, 1X, 3X, 5X, respectively) administered once daily for 26 weeks.

Route of Administration: Oral

Table 1: Description of Treatment Groups and Doses

Treatment Group	**Estriol Dose per Dog (mg)**	**Number of Tablets Given Orally Once per Day for 26 weeks**	**Number and Sex of Animals**
Placebo	0 mg	10 placebo tablets	6 female
1X	2 mg	2 estriol tablets	6 female
3X	6 mg	6 estriol tablets	6 female
5X	10 mg	10 estriol tablets	6 female

Variables Measured: Body weight, food consumption, clinical observations (including capillary refill time, mucus membrane color, appearance of vulva +/- discharge, body temperature, heart rate, and respiratory rate), physical examination, clinical pathology (hematology, clotting times, clinical chemistry, and urinalysis), gross necropsy, and histopathology, including bone marrow evaluation. Histopathologic examination was first performed on all tissues from animals that received placebo and those that received 10 mg of estriol per day. Tissues from dogs in the 2 mg and 6 mg groups were only examined if the next-higher dose group had significant lesions.

Statistical Analysis: The study was conducted using a randomized complete block design. The animals were blocked by weight where each block contained four dogs, randomly assigned to each of the four treatments. In all analyses, the experimental unit was the individual animal.

For continuous variables measured repeatedly throughout the study, data were examined by using a linear mixed model for repeated measures. The pretreatment mean was used as a possible covariate. The minimum Akaike Information Criterion was used to select among compound symmetry, compound symmetry heterogeneous variance, spatial power structure, or unstructured covariance structures. The Kenward-Roger (KR) approximation was used for approximating denominator degrees of freedom when needed. Fixed effects were treatment, day and treatment by day; block was a random effect. Treatments were compared by day if the treatment by day interaction was significant ($p \leq 0.10$) or compared averaged over days if the treatment effect was significant but the interaction was not ($p \leq 0.10$). Ordinal variables were analyzed using Fisher's Exact Test performed by Day or Week across treatment group levels.

Each continuous variable from the postmortem evaluations (organ weights and ratios to terminal body weight) was evaluated using a mixed model with treatment as a fixed effect and weight blocks as the random effect. If there was a significant treatment main effect ($p \leq 0.10$), then the comparisons of the treatments to the non-treated control were evaluated.

Results: Over the 26-week period, there was an increased incidence of abnormal clinical observations related to the estrogenic effects of estriol. There were more observations of redness and swelling of the vulva, vulvar discharges, and mammary hyperplasia for the estriol-treated dogs than the placebo-treated dogs. All dogs in the estriol-treated groups developed vulvar discharges (often reported daily) over the course of the study, whereas only 2 of 6 placebo-treated dogs did (a single observation in 1 dog and 2 observations in a second placebo group dog). Mammary hyperplasia was reported in 1 dog treated with placebo (2 observations), 5 of 6 dogs treated with 2 mg (2 observations for 1 dog and multiple observations for 4 dogs), and all dogs treated with 6 mg and 10 mg of estriol per day (multiple observations).

Dogs treated with 6 mg and 10 mg of estriol per day had higher white blood cell counts, absolute neutrophil counts, and platelet counts than dogs that received placebo. There were no estriol-related changes on bone marrow smears evaluated microscopically from dogs in the 10 mg group; therefore, bone marrow smears from dogs in the 2 mg and 6 mg groups were not

examined. The myeloid:erythroid (M:E) cell ratios were comparable between dogs that received 10 mg of estriol per day and those treated with the placebo.

Gross necropsy findings are summarized in Table 2 below. All dogs treated with estriol had enlarged vulvas except one dog that received 2 mg per day. There were miscellaneous kidney abnormalities observed in 3 dogs treated with 2 mg and 1 dog treated with 6 mg of estriol per day.

Table 2: Summary of Selected Gross Necropsy Findings

Gross Necropsy Finding	**Treatment Group and Number of Dogs**			
	placebo (0) n=6	**2 mg (1X) n=6**	**6 mg (3X) n=6**	**10 mg (5X) n=6**
Vulva				
Enlarged	0	5	6	6
Kidneys				
Depression, Deformity	0	2	0	0
Thickened	0	0	1	0
Enlarged	0	1	0	0

Table 3 below summarizes histopathology findings. Microscopically, vulvas and vaginas had a proestrus-like appearance, with or without a lymphoplasmacytic infiltrate. The findings in the vulva and vagina were attributed to the estrogenic activity of estriol. In the kidney, increased lymphoplasmacytic infiltrate in the pelvis and hyperplasia of the urothelium lining the pelvis were only observed in dogs treated with estriol; these findings were thought to represent an inflammatory reaction in the pelvis of the kidney. The incidence of abnormal findings in the kidney was low (one third or less of dogs in each estriol-treated group) and did not appear dose-related, but a relationship with estriol treatment could not be ruled-out. One dog in the 10 mg group had urinary bladder inflammation.

Table 3: Summary of Selected Histopathology Findings

Histopathology Finding	Treatment Group and Number of Dogs			
	placebo (0) n=6	2 mg (1X) n=6	6 mg (3X) n=6	10 mg (5X) n=6
Vulva				
Infiltrate, lymphoplasmacytic	NE[1]	1	4	5
Proestrus-like appearance	NE[1]	5	6	6
Vagina				
Infiltrate, lymphoplasmacytic	0	2	3	3
Proestrus-like appearance	0	3	6	6
Intermediate appearance	1	3	0	0
Anestrus-like appearance	5	0	0	0
Kidneys				
Infiltrate, lymphoplasmacytic, pelvis, increased	0	2	2	2
Hyperplasia, urothelium, pelvis	0	1	1	2
Urinary Bladder				
Inflammation	0	NE[1]	0	1

[1]NE = Not examined; histopathology not performed

Conclusions: Estriol administered orally at doses up to 10 mg per dog per day (5 times the proposed maximum daily dose) for 26 weeks to healthy, ovariohysterectomized female dogs approximately 1 year of age had an acceptable margin of safety. Estrogenic effects (swollen vulva, vulvar discharge, mammary hyperplasia) were associated with the administration of estriol.

B. Other Safety Observations:

In foreign, post-market pharmacovigilance data for estriol collected from the United Kingdom, France, Germany, the Netherlands, Switzerland, and New Zealand between the years 2000 and 2010 by Intervet, Inc., approximately 20% of reported adverse reactions were for local or general alopecia. Other adverse reactions reported in a very small number of dogs from these data and from extended-use studies in the United States were hyperpigmentation and lichenification of the vulva, vaginal hemorrhage, anemia, leukopenia and thrombocytopenia, possible uterine stump pyometra, and increase in epileptic seizures. Three dogs receiving estriol in US extended-use studies were euthanized due to aggressive behavior after 180, 306, and 320 days of therapy, respectively.

IV. HUMAN FOOD 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, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to INCURIN:

"Not for human use. Keep out of the reach of children. Women who are of child-bearing age or those who are breastfeeding should use caution when administering INCURIN Tablets. Wash your hands with soap and water after administration to avoid exposure to the drug. Consult a physician in case of accidental ingestion by humans."

VI. 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 21 CFR part 514. The data demonstrate that INCURIN, when used according to the label, is safe and effective for the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs.

A. Marketing Status:

The drug is restricted to use by or on the order of a licensed veterinarian because professional veterinary expertise is required to diagnosis urinary incontinence and to determine that the dog's condition is not due to other medical or surgical reasons.

B. Exclusivity:

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

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.