I. GENERAL INFORMATION

A. File Number

NADA 141-035

B. Sponsor

Ciba Animal Health, Ciba-Geigy Corporation
P.O. Box 18300
Greensboro, NC 27419-8300

C. Proprietary Name

PROGRAM® Tablets

D. Established Name

lufenuron tablets

E. Dispensing Status

Rx: For use by or on the order of a licensed veterinarian

F. Dosage Form, Route of Administration, and Recommended Dosage

The ingredients of PROGRAM Tablets are formulated into various sized tablets to be administered orally (swallow) as appropriate for the weight of the dog (see below) at monthly dosing intervals. The tablets supply the minimum recommended dose level of 10 mg lufenuron per kilogram of body weight.

Dog Weight	No. Tablet(s) Per Month	mg Lufenuron Per Tablet	Color
Up to 10 lbs.	1	45.0	Brown
11 to 20 lbs.	1	90.0	Red
21 to 45 lbs.	1	204.9	Yellow
46 to 90 lbs.	1	409.8	White

Dogs over 90 lbs. are provided the appropriate combination of these tablets.

G. Species/Class

Dogs, six weeks of age and older

H. Indication

PROGRAM Tablets are indicated for use in dogs, six weeks of age and older, for the prevention and control of flea populations.

II. EFFECTIVENESS

The new animal drug application for lufenuron tablets contains adequate and well-controlled studies which demonstrate efficacy in preventing and controlling flea populations. Lufenuron is an insect development inhibitor which breaks the flea life cycle at the egg stage. The adult female flea is exposed to the drug when feeding on

a treated dog. The drug, which has no deleterious effect on the adult flea, acts to inhibit the development of flea eggs. The mode of action is interference with the synthesis, polymerization and deposition of chitin, the major supportive component of the flea egg case and cuticle that forms the exoskeleton of larval stages.

A. DOSE ESTABLISHMENT

Three pivotal studies, one titration and two confirmation, were conducted to establish and confirm the minimum effective dose of lufenuron for the prevention and control of flea populations. These studies titrated and confirmed a monthly dose of 10 mg/kg body weight for dogs. A list of the pivotal dose establishment studies, principal investigators and study sites can be found in Table 1 .

PIVOTAL DOSE TITRATION STUDY: BPU-D-OH-022

Purpose: Dose Titration

Investigator:

Fred Hink, PhD
Ohio State University
Columbus, Ohio

Type of Study: Experimental infestations of the cat flea, *Ctenocephalides felis C. felis* is the common flea of both dogs and cats in most areas of the Unites States, with *C. canis* being found only occasionally. (Muller, GH, Kirk, RW, and Scott, DW. Small Animal Dermatology. W. B. Saunders Company, Philadelphia, 1989. pp. 347-426). In a field study of 3,000 fleas collected from dogs, 89% of the fleas collected were *C. felis* while only 11% were *C. canis* . (Dryden, M.W. Evaluation of Certain Parameters in the Bionomics of *Ctenocephalides felis felis*. Masters Thesis, Purdue University, West Lafayette, IN, 1988). Based on this information, the cat flea was used in all laboratory efficacy studies for lufenuron.

Animals: Forty adult dogs, 24 beagles and 16 mixed breeds, divided into 4 groups of 10 dogs each. Equal numbers of male and female dogs were used.

Controls: One group of 10 dogs receiving placebo tablets comprised of the formulation excipients without active ingredient.

Dosage Form: Lufenuron tablets (swallow)

Route of Administration: Orally, on a full stomach

Doses Tested: 5.0 mg, 10.0 mg, and 20.0 mg per kg animal body weight

Frequency of Treatment: One treatment

Duration of Study: Dogs were experimentally infested with newly emerged cat fleas weekly from day 7 to day 28. The dogs were treated on day 0. Flea eggs were collected from each dog twice weekly from day 3 to day 32 post-treatment. The

number of adult fleas emerging from these eggs were counted 35 days after they were collected.

Results: Efficacy was calculated by comparing the development of eggs collected from fleas feeding on treated versus control animals. The data provided below is an average from eggs collected on days 28 and 32 post-treatment:

Dose Group (mg/kg)	**Mean % Developmental Success Days 28 and 32**	**Mean % Control Days 28 and 32**
0 (Placebo)	58.80	
5	8.55	85.46
10	1.09	98.30
20	2.50	95.75

Note: Results were derived from the following calculations:

% Developmental Success = No. Adults Emerged / No. Eggs Collected x 100

% Control = Mean % Dev. Success(Control) - % Dev. Success(Treated) / Mean % Dev. Success (Control) x 100

Lufenuron at all doses tested provided >90% prevention of flea egg development through day 21 of the study. Since the drug is administered monthly, the critical parameter was determined to be efficacy at the end of the test period. Therefore, the combined efficacy from days 28 and 32 (above) was used to establish the dose of lufenuron. Efficacy was based on percent efficacy and no statistical analysis was required.

Conclusions:

The dose of 10 mg lufenuron per kg body weight provided >90% prevention of flea egg development and worked better than the highest dose used in the study. The lowest dose used in this study, 5 mg/kg, failed to prevent >90% egg development during the critical test period (days 28 and 32).

Adverse Reactions: No adverse reactions were reported.

PIVOTAL DOSE CONFIRMATION STUDY: BPU-D-OH-032

Purpose: Dose Confirmation

Investigator:

Fred Hink, PhD
Ohio State University
Columbus, Ohio

Type of Study: Experimental infestations of the cat flea, *Ctenocephalides felis*

Animals:

Twenty adult beagle dogs divided into two groups of 10 dogs each. Equal numbers of male and female dogs were used.

Controls:

One group of 10 dogs received placebo tablets comprised of the formulation excipients without active ingredient.

Dosage Form: Lufenuron tablets (swallow)

Route of Administration: Orally, on a full stomach

Dose Tested:

Minimum recommended dose of 10 mg per kilogram animal body weight administered to one group of ten dogs, 5 males and 5 females.

Frequency of Treatment: One treatment

Duration of Study:

Dogs were infested with newly emerged cat fleas on days 7, 5, 0, and approximately every week, thereafter. The dogs were treated on day 0. Eggs were collected from each dog twice weekly from day 7 to day 32 post-treatment. The number of adult fleas emerging from these eggs were counted 35 days after they were collected.

Results:

Efficacy was calculated by comparing the development of eggs collected from fleas feeding on treated versus control animals. The data provided below is an average from eggs collected on days 28 and 32 post-treatment.

Dose Group (mg/kg)	**Mean % Developmental Success Days 28 and 32**	**Mean % Control Days 28 and 32**
10	0.05	99.9
0	68.55	NA

Note: Results were derived from the following calculations:

% Developmental Success = No. Adults Emerged / No. Eggs Collected x 100

% Control = Mean % Dev. Success(Control) - % Dev. Success(Treated) / Mean % Dev. Success (Control) x 100

Conclusions: Lufenuron, administered at a minimum of 10 mg/kg body weight, was effective (>90%) in preventing flea egg development measured during the critical end of the month (days 28 and 32) period.

Adverse Reactions: No adverse reactions were reported.

PIVOTAL DOSE CONFIRMATION STUDY: BPU-D-AL-034

Purpose: Dose Confirmation

Investigator:

Byron Blagburn, PhD
Auburn University
Auburn, AL

Type of Study: Experimental infestation of the cat flea, *Ctenocephalides felis*

Animals:

Twenty-four 7 month old female beagle dogs (female dogs were used based on availability).

Controls:

Placebo tablets, comprised of formulation excipients without active ingredient

Dosage Form: Lufenuron tablets (swallow)

Route of Administration: Orally, on a full stomach

Dosage and Frequency of Treatment:

Minimum recommended dose of 10 mg per kg of animal body weight administered to four groups of three dogs each on study days 7, 37, and 68. Four groups of 3 dogs each were treated with the placebo tablets on study days 7, 37, and 68.

Duration of Study: 91 days

Study Design:

Dogs were housed in two adjacent buildings, each divided into 4 rooms measuring 4' by 7'. Each room was paneled and carpeted so as to simulate a typical home environment and had access to outside runs. Each room housed 3 dogs from the same treatment group.

On study days 0 and 2, all dogs were infested with newly emerged cat fleas. On day 6, all dogs were combed and the fleas enumerated and returned to their respective canine hosts. The carpets were seeded with pulverized dog food, dried beef blood,

brewer's yeast and sand to provide food for developing flea larvae. Weekly from day 14 to day 91, the dogs were combed, fleas enumerated, and returned to their respective canine hosts.

Results: Total adult flea counts were made weekly to measure drug efficacy.

Test Day	Mean Flea Counts Treated Groups	Mean Flea Counts Control Groups	% Control
2	22.3	20.3	0
*7	76.6	72.6	0
14	29.1	31.6	8
21	64.5	120.1	46
28	44.8	221.1	80
35	17.4	184.8	91
42	10.9	262.1	96
49	6.9	141.2	95
56	9.1	208.4	96
63	6.1	147.4	96
70	2.3	190.0	99
77	6.4	145.3	96
84	2.9	123.3	98
91	5.0	52.0	90

*Treatment given

Note: Results were derived from the following calculation:

% Control = Mean No. Fleas (Control) - Mean No. Fleas (Treated) / Mean No. Fleas (Control) x 100

Conclusions: In a simulated home environment, lufenuron provided >90% control of flea infestations when administered at the minimum use rate of 10 mg/kg body weight for the last 2 months of a 3 month treatment period. Because the dogs in this study were infested with fleas prior to treatment and because lufenuron has no effect on the adult fleas, there was a 35 day lag period before the drug's effect on the flea life cycle could impact the adult flea population.

Adverse Reactions:

No adverse reactions to treatment were reported. Seven dogs in the control group and 1 dog in the treated group showed dermatologic signs attributable to flea bites or flea allergic dermatitis. Diarrhea was noted in 4 runs (1 treated and 3 control) and fecal analyses were positive for *Giardia* sp.

CORROBORATIVE DOSE CONFIRMATION STUDY: BPU-D-IL-039

Investigator:

Ron Smith, PhD
University of Illinois
Urbana, IL

Animals: Sixteen adult female beagle dogs

Study Design/Dosage/Frequency of Treatment:

Control and treated dogs were maintained in identical indoor/outdoor runs. Indoor runs were carpeted and maintained at normal temperature and humidity to simulate a home environment. All animals were experimentally infested with fleas at the onset of the study. Lufenuron was administered at the minimum recommended dose of 10 mg/kg body weight to two groups of 4 dogs. Dosing took place on days 7, 37, and 67. Placebo tablets were administered to two groups of 4 dogs on days 7, 37, and 67. The dogs were combed weekly until day 70, the fleas on each dog were counted, and returned to their respective canine hosts.

Results:

An active flea infestation was not achieved in one of the two control groups. This group and its corresponding treated group (both housed on the west side of the building) were not included in the evaluation of efficacy. In the remaining lufenuron-treated group, 97% control of fleas was achieved by day 63 when compared to the remaining control group.

Conclusions:

In a simulated home environment, lufenuron provided >90% control of flea infestations when administered at the minimum use rate of 10 mg/kg body weight. Because the dogs in this study were infested with fleas prior to treatment and because lufenuron has no effect on the adult fleas, there was a lag period before the drug's effect on the flea life cycle had an impact on the adult flea population. The lag period was 63 days for this study conducted in Illinois compared to the 35 day lag period seen in study BPU-D-AL-034 conducted in Alabama. In cooler geographic areas where the life cycle of the flea is prolonged, the time required for control of an existing flea population may also be prolonged.

Adverse Reactions: No adverse reactions were reported.

TABLE 1

PIVOTAL DOSE ESTABLISHMENT STUDIES

Study Number	Investigator/Location	Doses Evaluated	Type of Study
BPU-D-OH-022	Dr. Fred Hink Ohio State University Columbus, OH	5, 10 & 20 mg/kg	Dose Titration
BPU-D-OH-032	Dr. Fred Hink Ohio State University Columbus, OH	10 mg/kg	Dose Confirmation
BPU-D-AL-034	Dr. Byron Blagburn Auburn University Auburn, AL	10 mg/kg	Dose Confirmation

B. WELL-CONTROLLED CLINICAL FIELD TRIALS

Lufenuron tablets were evaluated in a multi-centered, well-controlled clinical field trial conducted in twenty-one veterinary clinics located in several geographic locations. Each study site adhered to one of two study regimen protocols; Regimen I evaluated lufenuron tablets in animals with low or no existing flea infestations or Regimen II which evaluated lufenuron tablets in animals with pre-existing flea infestations. A total of 411 dogs were enrolled in Regimens I and II of the clinical trials and a total of 306 of the dogs enrolled were accepted for inclusion in the analysis. Animals were excluded from the data base for a number of reasons, primarily for protocol violations and owner non-compliance. A list of the clinical investigators participating in Regimens I and II can be found in Table 2

The following table displays the numbers of dogs and households that were enrolled and found acceptable for analysis (parentheses) in the study:

	Dogs	**Households With Dogs**
Lufenuron	214 (155)	122 (91)
Control (Placebo)	197 (151)	117 (94)

The age and weight ranges of the dogs included in the data analysis are provided below:

Weight	**Lufenuron**	**Control**
Up to 10 lbs.	16	12
11 to 20 lbs.	26	29
21 to 45 lbs.	39	44
46 to 90 lbs.	69	57
> 90 lbs.	5	9

Age	**Lufenuron**	**Control**
< 6 months	3	4
6 months to 10 years	120	134
> 10 years	32	13

Only 3 dogs < 6 months of age were treated with lufenuron in the study. Data to support the use of lufenuron in dogs < 6 months of age were generated in the pivotal target animal safety studies (see pages 14-28). In Pivotal Studies 1 and 2 (see pages 14-17), the dogs were 8 weeks of age when treated with lufenuron was initiated. In Pivotal Study 5 (see page 20) the dogs were 6 weeks of age at the start of the study. Approximately 44 different breeds of dogs were enrolled in the clinical trials (34 included in the analysis) as well as a large and diverse selection of mixed breeds.

During the course of the trials, no treatment related deaths or adverse reactions were attributed to lufenuron. Isolated cases of vomiting occurred but were not consistent for any specific animal, breed, dose, treatment or placebo group, and, therefore, not attributed to lufenuron. Lufenuron was administered concurrently with a wide range of routinely used veterinary medications and vaccines without any adverse reactions reported.

Regimen I

Type of Study:

Two-group, double-blind, placebo-controlled study designed to evaluate the effect of lufenuron tablets under "pre-infestation" conditions.

Test Groups: Group A received lufenuron tablets and Group B received placebo tablets.

Number of Veterinary Clinics Participating: Eleven

Animals:

Each investigator was to recruit at least 12 households for the study which were randomly assigned to one of the two treatment groups. All animals from a household were enrolled in the same treatment group.

Study Duration: Minimum of six months.

Dosage Administration:

Lufenuron tablets, formulated to provide the minimum effective dose of 10 mg/kg, were administered by the dog owners to dogs enrolled in Group A once-a-month for a minimum of six consecutive months. In clinical field trial locations experiencing a "light" flea season (as determined by the clinical investigators based on their experience), the study protocol was amended to extend the study past six months.

Controls:

Placebo tablets, formulated without active ingredient, were administered by the dog owners to Group B animals once-a-month for six consecutive months or longer if a particular study site used an extended protocol.

Clinical Evaluation:

At enrollment all animals were given a complete physical examination and blood was collected for a complete blood count (CBC) and serum chemistry profile. Flea counts were performed in the clinic prior to treatment and monthly, thereafter, for the duration of the study. A short-acting adulticide (Adams(TM) Flea & Tick Mist) was used on dogs from both groups as labeled once-a-month to kill adult fleas for removal and counting. At the conclusion of the study, a final flea count and physical exam were performed. Blood was also collected for a CBC and serum profile.

Concomitant Therapy:

Other than once-a-month administration of the investigational drug and placebo and the adulticide used at the veterinary clinic for flea counting, no other flea treatments to animals or their environments were allowed. Other treatments and medications commonly used in the practice of veterinary medicine were allowed.

Results: Regimen I

In Regimen I, treatment with lufenuron was initiated in animals before the onset of flea infestations. Of the 213 dogs enrolled under this regimen, 107 comprised the lufenuron treatment group and 106 the placebo treatment group. These animals represented a total of 127 dog households, 64 and 63 in the lufenuron and placebo groups, respectively. A total of 173 cases were included in the data analysis; 89 comprised the lufenuron treatment group and 84 the placebo treatment group. A total of 156 cases completed the trial; 85 in the lufenuron treatment group and 71 in the placebo treatment group. The number of dogs initially enrolled, included in the data analysis, and remaining in the study at four and six months are provided below:

			Dogs In Trial At	
Group	**Enrolled**	**Analyzed**	**4 Months**	**6 Months**
A. Lufenuron	107	89	89	85
B. Control	106	84	83	71

The study design allowed those households which developed intolerable flea infestations to withdraw from the study after the fourth month exam. This was necessary especially for Group B (placebo control) where severe flea infestations developed without adequate control measures. Some households dropped from the study for other reasons including convenience in adhering to the protocol. Data analysis was performed only on data through the six month exam to prevent the data from becoming skewed by households with heavier flea infestations dropping from the study and households with fewer fleas remaining on trial. Descriptive statistics presented for all evaluation time points through six months are presented below for both lufenuron and placebo treated groups:

Descriptive Statistics Regimen I

Month	**Number of Dogs**	**Mean Flea Counts**
Group A/Lufenuron		
0	89	1.01
1	89	1.96
2	89	0.91
3	89	2.54
4	89	3.46
5	87	4.02
6	85	4.44
Group B/Placebo		
0	84	1.06
1	84	3.38
2	84	7.26
3	84	24.15
4	83	48.92
5	77	99.77
6	71	230.86

Statistical Analysis: Non-parametric, one-way analyses of variance were used to analyze Savage scores, based on monthly flea counts. Dogs with 20 or fewer fleas at the study initiation were included in the analyses. The placebo and lufenuron treatment groups were compared at each time point. Significant differences in Savage scores were found between treatment groups from month 2 onwards ($p<0.05$). Average flea counts were lower in the lufenuron-treated dogs than in the placeob-treated dogs from month 1 onwards.

Conclusions:

Lufenuron, administered at a minimum dose of 10 mg/kg body weight at monthly intervals starting prior to the flea season, prevented the build-up of flea populations after approximately 60 days following the initiation of monthly dosing.

Regimen II

Type of Study:

Two-group, double blind, positive-controlled study designed to evaluate efficacy of lufenuron tablets in dogs with established flea infestations.

Test Groups:

Group C received lufenuron tablets plus adulticide treatment (Adams(TM) Flea & Tick Mist) and Group D received placebo tablets plus a topical adulticide and insect growth regulator (Ovitrol Plus[reg.]---an Environmental Protection Agency-regulated topical insecticide). See Clinical Evaluation for further explanation.

Number of Veterinary Clinics Participating: Ten

Animals:

Each investigator was to recruit at least 12 households for the study which were randomly assigned to one of the two treatment groups. All animals from a household were enrolled in the same treatment group.

Study Duration: Minimum of 6 months.

Dosage Administration:

Lufenuron tablets, formulated to provide the minimum effective dose of 10 mg/kg, were administered once-a-month by the dog owners for a minimum of six consecutive months to dogs enrolled in Group C. In clinical field locations experiencing a "light" flea season (as determined by the clinical investigators based on their experience), the study protocol was amended to extend the study past six months.

Controls:

The control (D) group in this trial received a placebo administered by the dog owner in a dosage form identical to lufenuron tablets, as well as treatment with a positive

control insect growth regulator (Ovitrol Plus®) per label instructions by the veterinary staff of the veterinary clinic.

Clinical Evaluation:

At enrollment, all animals were given a complete physical examination and blood was collected for a CBC and serum chemistry profile. As an initial treatment for the existing flea infestations and to assure compliance in the early stages of the study when flea infestations were likely to be the heaviest, households from both groups used weekly, on-animal pyrethrin adulticide applications (Adams(TM) Flea & Tick Mist) for the first eight weeks of the study. Flea counts were performed in the clinic prior to treatment and monthly, thereafter. A short acting adulticide (Adams(TM) Flea & Tick Mist) was used as labeled once-a-month at the veterinary clinic in the lufenuron-treated group to kill fleas for removal and counting. The control group received Ovitrol® Plus, which contains an adulticide (pyrethrin) and an insect growth regulator (methoprene), once-a-month at the veterinary clinic to kill adult fleas for removal and counting. The insect growth regulator in the product served as the positive control for the study. At the conclusion of the study, a final flea count and physical exam was performed. Blood was also collected for a CBC and serum profile.

Concomitant Therapy:

Other than those treatments mentioned above, no other flea treatments to animals or their environment were allowed. Other types of medications commonly used in veterinary practice were allowed as needed.

Results: Regimen II

In Regimen II, lufenuron tablets were evaluated in animals with pre-existing infestations of fleas. Of the 198 dogs enrolled under this protocol regimen, 107 comprised the lufenuron treatment group and 91 the control group. These animals represented a total of 112 households; 58 in the lufenuron group and 54 in the control group. A total of 133 cases were included in the analysis; 66 comprised the lufenuron treatment group and 67 the control group. All dogs included in the analysis completed the trial.

Group	Enrolled	Analyzed	Dogs In Trial At 6 months
C. Lufenuron	107	66	66
D. Control	91	67	67

Descriptive statistics presented for all evaluation time points through six months are presented below for both lufenuron and control groups:

Descriptive Statistics Regimen II

Month	**Number of Dogs Group C/Lufenuron**	**Mean Flea Counts**
0	66	74.12
1	66	31.50
2	66	10.58
3	66	11.08
4	66	8.59
5	65	8.32
6	66	4.05

Descriptive Statistics Regimen II (Continued)

Month	**Number of Dogs Group D/Control**	**Mean Flea Counts**
0	67	78.25
1	66	32.55
2	67	4.54
3	67	2.10
4	66	2.26
5	67	2.18
6	67	1.25

Statistical Analyses: Non-parametric, one-way analyses of variance were used to analyze Savage scores, based on monthly flea counts. Dogs with as few as one flea at the study initiation were included in the analyses. The positive control and lufenuron-treatment groups were compared at each time point. No significant differences in Savage scores were found between treatment groups at any time point ($p>0.05$), though average flea counts were higher in the lufenuron-treated dogs than in the positive control-treated dogs from month 2 onwards.

Conclusions:

Lufenuron, administered at a minimum dose of 10 mg/kg body weight at monthly intervals during active flea infestations, effectively controlled the flea populations. Lufenuron, an insect development inhibitor which has no effect on the adult flea, was used in combination with a topical insecticide to kill the adults for the first 2 months of the study when the pre-existing flea infestations were at their heaviest. The investigational insect development inhibitor (lufenuron), administered by the dog owners, worked as effectively as the insect growth regulator in the topical spray, Ovitrol® Plus, applied by the veterinary staff.

Adverse Reactions: No adverse reactions were reported.

TABLE 2 CLINICAL INVESTIGATORS

REGIMEN I

Dr. Bill Bledsoe Spartanburg, SC	Dr. Bill Paramore Carmel, IN
Dr. Jere Colley Dr. Gary Hunt Opelika, AL	Dr. Ann Parker Fayetteville, NC
Dr. Richard Devries Albany, NY	Dr. Ken Schoolmeester Dr. Karen Kennedy Greensboro, NC
Dr. M. K. Jacobsen Indianapolis, IN	Dr. Virgil Tongish Westerville, OH
Dr. Joe Kinnarney Reidsville, NC	Dr. Todd Schadler Columbus, OH
Dr. Walter Legg Lewisville, TX	

REGIMEN II

Dr. Maynard Clark Lafayette, CA	Dr. Perry Smith Miami, FL
Dr. Bill Craig San Antonio, TX	Dr. Jan Strother Hartselle, AL
Dr. Dan McIlhany San Antonio, TX	Dr. Tim Sung Hercules, CA
Dr. Jim Raab Fort Pierce, FL	Dr. Herb Utgard North Miami Beach, FL
Dr. Doug Riley Arlington, TX	Dr. Charles Ward Carrboro, NC

III. TARGET ANIMAL SAFETY

Nine pivotal target animal safety studies were conducted in dogs to address the tolerance and safety of lufenuron. These studies were specifically designed to evaluate safety of the drug administered at exaggerated doses in breeding animals, in a long-term (10-month) study, in a study with commercially available flea adulticides and in six-week old puppies. These studies demonstrated that lufenuron tablets provide a wide therapeutic index when administered orally to dogs at the minimum recommended dose of 10 mg/kg body weight, monthly. A list of the target animal safety studies, principal investigators and study sites can be found in Table 3.

Pivotal Study 1:

Ten-Month Oral Toxicity Study In Beagle Dogs

Type of Study:

A chronic (10 month) study during which lufenuron tablets were administered at 0, 1X, 3X, or 5X the monthly use rate of 10 mg/kg body weight daily for three consecutive days each month from months one to three and at 0, 2X, 6X, and 10X for three consecutive days each month from months four through ten of the study.

Investigator/Study Director:

Dr. Edwin Goldenthal
International Research and Development Corp. (IRDC)
Mattawan, MI

The dogs used in this study were whelped at Hazelton Laboratories, Michigan. During the tenth week of the study, when they were 18 weeks of age, the dogs were transported to IRDC.

Animals:

Twenty-four male and twenty-four female purebred beagle dogs that entered the study at eight weeks of age and were mature (approximately one year of age) at the conclusion of the study.

Dosage Form: Lufenuron Tablets (swallow)

Route of Administration: Oral (at IRDC, dogs were fed at 7 am and were dosed within 2 hours).

Dosage and Frequency of Treatment:

The dogs were assigned to 4 groups of 12 dogs each, six males and six females. Three groups were dosed at 1X, 3X and 5X the recommended use rate of 10 mg/kg body weight. These dosages were administered on three consecutive days per month for 3 months. For the following seven months, dosages were doubled from 1X to 2X, 3X to 6X and 5X to 10X, respectively. The reason for this dosage increase was to show safety at exaggerated doses since the proposed dosage scale allows for some degree of overdosage at the low end of each weight range.

Controls:

Twelve animals each, six male and six female, were dosed with placebo tablets in a manner identical to the lufenuron treatment groups.

Study Duration: Ten Months

Evaluation:

Criteria evaluated for treatment effect included observations for overt toxicity, moribundity and mortality, body weights, food consumption, water consumption, ophthalmologic examinations, hematological, biochemical and urinalysis determinations, and macroscopic and microscopic examination of tissues, including selected major organ weights. All surviving dogs were sacrificed following ten months of study.

Results:

One female dog in the 1X dose group died on study day 16 due to pneumonia and was replaced with another female dog. The death was determined to be unrelated to the administration of the test article. At the 1X (2X) use rate, all criteria evaluated were considered to be comparable to findings in the control group. At the 3X (6X) and 5X (10X) use rates all criteria evaluated were considered to be comparable to findings in the control group with the exception of the average food consumption. Females in the two highest dose groups showed a decrease in average food consumption compared to the control females, as shown in the following table:

AVERAGE MEAN GRAM/ANIMAL/DAY FOOD DOSAGE CONSUMPTION VALUES WITH THE % LEVEL DIFFERENCE FROM THE CONTROL GROUP

	MALES	FEMALES
0	321	323
1X (2X)	338 (+5.3)	334 (+3.4)
3X (6X)	324 (+0.9)	279 (-13.6)
5X (10X)	360 (+12.1)	254 (-21.4)

The decreased food consumption in the 3X (6X) and 5X (10X) females had little effect on body weight, as shown in the following table:

DOSAGE MEAN BODY WEIGHTS (KG) AT WEEK 44 WITH THE LEVEL PERCENT DIFFERENCE FROM WEEK 1

	MALES	FEMALES
0	12.6 (+641)	9.5 (+533)
1X (2X)	11.6 (+544)	10.2 (+580)
3X (6X)	11.9 (+526)	10.2 (+467)
5X (10X)	12.0 (+531)	9.1 (+435)

Conclusions: There were no overt signs of toxicity when lufenuron was administered to 8 week old puppies at doses up to 5X the intended monthly use rate for three consecutive days per month for 3 months and to 5 month old puppies at doses up to 10X the intended monthly use rate for three consecutive days per month for 7 months. Decreases in food consumption were evident in the female dogs given lufenuron at 3X (6X) and 5X (10X) the use rate, though there was little effect on the mean body weight of these dogs at termination.

Pivotal Study 2:

Six-Month Oral Toxicity Study In Beagle Dogs With Lufenuron In Combination With Commercial Flea Insecticides

Type of Study:

A study designed to evaluate the safety of lufenuron when used simultaneously with commercially available flea adulticides.

Investigator/Study Director:

Dr. Edwin Goldenthal
International Research and Development Corp. (IRDC)
Mattawan, MI

The dogs used in this study were whelped at Hazelton Laboratories, Michigan. During the tenth week of the study, when they were 18 weeks of age, the dogs were transported to IRDC.

Animals:

Eighteen male and eighteen female purebred beagle dogs entered the study as weanlings at eight weeks of age. The six groups used in the study contained three male and three female dogs each.

Dosage Form: Lufenuron Tablets (Swallow)

Controls: Placebo Tablets (Swallow)

Dosage/Frequency of Treatment:

Lufenuron tablets were administered at 10X the recommended use rate (10 mg/kg monthly) for the first three days each month for six consecutive months. Commercially available insecticides which have activity against the adult flea were administered concurrently to these dogs. These insecticides were used according to label recommendations, with the exception of chlorpyriphos (group III below) which is not labeled for use in dogs less than 4 months of age. The study evaluated safety parameters in dogs receiving the following treatment regimes:

Group	**Lufenuron Treatment**	**Commercial Insecticide**
Control	0 (Placebo)	None
I	10X	Carbaryl/Once Weekly
II	10X	Permethrin/Every Two Weeks
III	10X	Chlorpyriphos/Every Three Weeks
IV	10X	Cythioate/Twice Weekly
V	10X	None

The commercial insecticides were administered beginning within 2 days after completion of the first monthly dose of lufenuron. The carbaryl product (Mycodex® Powder Plus) was applied as a topical powder, the permethrin (Expar(TM)3.2%EC) and chlorpyriphos (Dursban® Dip) were applied topically as dips and the cythioate (Proban®) as an oral tablet. During week 4 of the study, the dogs were not dosed with any of the above treatments due to a *Giardia* infestation. All dogs were treated with metronidazole (Flagyl®) for 5 days.

Study Duration: Six Months

Evaluation:

Criteria evaluated for treatment effect included observations for overt toxicity, moribundity and mortality, body weights, food consumption, ophthalmologic examination, hematological, biochemical and urinalysis determination, and macroscopic and microscopic examination of tissues, including selected major organ weights. All surviving animals were sacrificed following six months of study.

Plasma cholinesterase and red blood cell (RBC) cholinesterase were determined pretest and monthly, thereafter.

Results:

There were no opthalmological, hematological, urological, macroscopic or microscopic pathological abnormalities found in any of the groups. Food consumption, body weight, and organ weights at necropsy were normal for all groups.

Slight hair loss/alopecia was observed in 27 of the 36 dogs in this study, including 3 of the 6 control dogs. It's significance is unknown. One dog in group I was sacrificed on study day 2 due to poor condition. Gross necropsy revealed a thin, dehydrated dog with pale mucous membranes. Based on the chronic nature of the presenting clinical signs, the dog was presumed to have been ill at the initiation of the study.

Cholinesterase was inhibited in those groups exposed to organophosphates (chlorpyriphos and cythioate); however, no clinical signs typical of organophosphate toxicity (vomiting, diarrhea, weakness, salivation, muscular tremors) were observed. Plasma cholinesterase values were reduced at the 6-month interval of analysis in the group treated with both lufenuron and chlorpyriphos (group III). A reduction in plasma cholinesterase was also noted in females in this group at the 3-month analysis interval. An additional study was conducted to determine if this effect was due to lufenuron or chlorpyriphos (see Pivotal Study 3).

A reduction in red blood cell cholinesterase was observed in females treated with lufenuron and cythioate (group IV) at the third and fourth month analysis intervals. Since the five and six month values were not different from controls, this effect was considered transient and attributable to cythioate.

Conclusions:

No overt signs of toxicity were observed in dogs administered lufenuron at 10X the use rate for three consecutive days per month for 6 consecutive months in combination with commercially available flea adulticide products used at their labeled use rate. This study demonstrates that lufenuron, when used concomitantly with common flea adulticide products, does not result in any additional side effects; however, plasma cholinesterase inhibition in dogs exposed to lufenuron plus chlorpyriphos was observed. This inhibition is addressed in an additional study (see Pivotal Study 3).

Pivotal Study 3:

Six-Week Oral Toxicity Study In Dogs With Lufenuron In Combination With Chlorpyriphos

Type of Study:

To define the reduced plasma cholinesterase values observed in Pivotal Study 2: Six-Month Oral Toxicity Study In Beagle Dogs With Lufenuron In Combination With Commercial Flea Insecticides.

Investigator/Study Director:

Dr. Edwin Goldenthal
International Research and Development Corp.
Mattawan, MI

Animals:

Twelve male and twelve female purebred beagle dogs entered the study at approximately 5-6 months of age. Four study groups contained three male and three female dogs each.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Untreated

Dosage/Frequency of Treatment:

Lufenuron tablets were administered at 100 mg/kg, 10X the recommended monthly use rate (10 mg/kg) for the first three days of weeks 1 and 5 of the study. The four groups evaluated in this study are provided below. Chlorpyriphos was administered once weekly for the first three weeks of the study.

Group	**Lufenuron Treatment**	**Chlorpyriphos Treatment**
I	None	None
II	None	Weekly/For Three Weeks
III	100 mg/kg/For Three Days	Weekly/For Three Weeks
IV	100 mg/kg/For Three Days	None

The recommended use rate of chlorpyriphos (Dursban® Dip) is once every 3 weeks. In this study, the product was applied topically as a dip once a week for three weeks in order to rapidly cause cholinesterase inhibition.

Study Duration: Six Weeks

Evaluation:

Criteria evaluated for treatment effects included observations for overt toxicity, moribundity, mortality, body weights, food consumption, hematological, biochemical and urinalysis determination.

Plasma and RBC cholinesterase were determined pretest and at weeks 2, 4, 5, and 6 of the study.

Results:

No findings were observed in any of the measured parameters except for plasma cholinesterase. Plasma cholinesterase levels were depressed in all groups exposed to chlorpyriphos. No differences were observed in the rate or degree of plasma cholinesterase depression between the chlorpyriphos-only and the lufenuron-chlorpyriphos groups. There was no difference in plasma cholinesterase levels between the lufenuron treated and control groups.

Conclusion:

Lufenuron does not cause cholinesterase inhibition nor does it enhance cholinesterase depression caused by exposure to organophosphates.

Pivotal Study 4:

Acute Oral Toxicity Study (Tolerability) In Dogs With Lufenuron

Type of Study:

The objective of this study was to evaluate the acute oral toxicity of lufenuron to dogs administered a single dose equivalent to 20X the recommended monthly use rate.

Investigator/Study Director:

Dr. Edwin Goldenthal
International Research and Development Corp.
Mattawan, MI

Animals:

Three male and three female, purebred beagle dogs, approximately 7-8 months of age.

Dosage Form: Lufenuron Tablets (swallow)

Controls: No controls were used.

Dosage/Frequency of Treatment:

Lufenuron was administered in a single dose by tablet combinations to achieve 200 mg/kg (20X the recommended dose).

Study Duration: Fifteen days post treatment.

Evaluation:

Mortality, moribundity, body weights, overt toxicity, hematological and biochemical parameters were the criteria evaluated for treatment effects.

Results:

No test article related hematological, biochemical or urological changes were observed. Clinical findings, common for laboratory-reared dogs, were occasional and transient. These included soft stools/diarrhea (5/6 dogs, lasting from 1 to 3 days), emesis (1/6 dogs on day 12), lacrimation (3/6 dogs through most of the study) and trembling (1/6 dogs on day 8). Mean body weights were lower at day 15 compared to pretest levels in both males and females.

MEAN BODY WEIGHT (kg)

	Females	Males
Pretest	10.9	11.2
Week 1	10.7	11.3
Week 2	10.7	11.4
Week 3	10.4	11.0

Conclusion: Lufenuron, when administered at 20X the recommended use rate did not demonstrate any marked toxic effects; however there was a downward trend in body weight exhibited in both males and females.

Pivotal Study 5:

90-Day Toxicity Study In Young Beagle Dogs (6-Weeks Or Less In Age) With Lufenuron

Type of Study:

To evaluate the safety of lufenuron when given to young puppies, six weeks of age or less, over a 90-day period of time.

Investigator/Study Director:

Martin Gilman, Ph.D.
William R. Voss, D.V.M.
Hazelton Research Products, Inc.
Kalamazoo, MI

Animals:

Twelve male and twelve female, purebred beagle dogs, six weeks of age or less (not having reached 49 days of age) at initiation were selected for use on study. The four evaluation groups in the study contained three male and female dogs each.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Placebo Tablets (swallow)

Dosage/Frequency of Treatment:

Each animal was dosed orally on three consecutive days per month for three months. The control group received placebo tablets equal to the largest lufenuron dose. Animals were randomly assigned to the following groups:

Group	Dose mg/kg	Multiples of Use Level
1	Placebo	0
2	10	1X
3	30	3X
4	50	5X

Study Duration: 90 Days

Evaluation:

Criteria evaluated for treatment effect included observations for overt toxicity, moribundity and mortality, body weights, food consumption, hematological, biochemical and urinalysis determinations.

Results:

Six week old puppies treated with lufenuron at up to 5X the use rate on three consecutive days per month for three months showed an increased incidence of dehydration and emesis compared to control puppies (see table below); however, all treated puppies grew at a normal rate and were healthy at the conclusion of the study.

CLINICAL SIGN	0X	1X	3X	5X
Dehydration	17%	50%	33%	67%
Emesis	33%	83%	83%	67%

Conclusions: Lufenuron, at up to 5X the recommended minimum use rate of 10 mg/kg is safe for use in 6 week old puppies; however, treated puppies may show an increased incidence of certain clinical signs, i.e., dehydration and emesis. These clinical signs do not affect the growth rate of treated puppies.

REPRODUCTIVE SAFETY/LABORATORY STUDIES (GLP)

Pivotal Study 6:

A Reproduction Study In Beagle Dogs With Lufenuron

Type of Study:

The objective of this laboratory study was to evaluate the effects of lufenuron on reproduction in the dog.

Investigator/Study Director:

Mr. James Schardein
International Research and Development Corp.
Mattawan, MI

Animals:

Twenty-five female and twenty-five male beagle dogs were selected from the stock population of proven dams and sires at Hazleton-Laboratory Research Enterprises, Inc., Kalamazoo, MI. Animals were a minimum of two years old and bitches had whelped at least two times previously. During an acclimation period, all dogs were dosed with the placebo for seven consecutive days and a semen evaluation was performed on all males. The five males and five females judged least suitable to fulfill study objectives, based on semen quality and/or tendency toward tablet regurgitation, were eliminated from the study. A total of twenty males and twenty females were selected for inclusion in the study.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Placebo Tablets (swallow)

Dosage/Frequency of Treatment:

To ensure maximum exposure during all phases of embryogenesis, lufenuron was administered daily at 3X the recommended monthly use rate of 10 mg/kg, equivalent to a minimum dosage of 30 mg/kg body weight/day (90X the minimum recommended monthly use rate). Control dogs received the corresponding number of placebo tablets. Males and females were dosed starting approximately three months prior to mating and continued throughout the mating period. Bred females were dosed throughout gestation, whelping, and weaning for a total of approximately nine months.

Study Groups/Mating:

Prior to dosing, animals were listed in descending order by body weights and assigned to one control and one treatment group of twenty animals each; ten studs and ten bitches. Within each study group, the first bitch with signs of proestrus was paired with the first stud etc., until all animals were paired.

Study Duration: Approximately nine months.

Evaluation:

Specific parental criteria evaluated were survival rate, appearance and behavior, body weights, stud sperm quality, stud and bitch fertility indices and mean gestation period. Viability, body weights, growth and survival indices were criteria measured for pups from the litters produced.

Results:

The ratio of gravid females to females mated was 8/8 or 100% in the control group and 6/9 of 66.7% in the lufenuron treated group. Comparing the study results to historical colony fertility data from Hazleton-LRE (86-88%), it was determined that no biological conclusions could be drawn from the difference in the fertility rate between control and treated groups. It could not be determined if this was a drug effect or a function of the small sample size (10 bitches per group) There were no treatment related effects for the other reproductive parameters measured, though one bitch in the treated group had a "possible" retained placenta. Any bitch with green discharge post-whelping is classified as having a possible retained placenta and is treated with oxytocin as a preventive measure. The diagnosis was not confirmed. The pertinent reproductive parameters measured are shown in the following table:

	Fertility Index gravid females/females mated	**Gestation Length** average (range)	**Whelping Index** # live pups/ # gravid females	**Weaning Index** # live pups day 42/ # live pups day 0
Control	8/8 (100%)	60.6 (57-63)	35/8 (4.4)	28/35 (80%)
Treated	6/9 (66.7%)	62.7 (59-69)	39/6 (6.5)	34/39 (87%)

The mean birth weight of pups from treated bitches was lower than from control bitches. It could not be determined if this was a drug effect or a function of litter size. The mean number of live pups per litter in the treated group (6.5) was two animals higher than the concurrent control (4.4). Treated pups in this study grew at a similar rate to both colony and concurrent controls.

Pup Mean Body Weights From Lactation Day 1 to Weaning (day 42) (grams)

	1	14	28	42
Control	324.9	854.3	1403.0	2132.3
Treated	278.1	620.8	1011.2	1661.8
Colony	293.9	719.8	1131.7	1796.0

There was a higher incidence in the treated versus control group pups of clinical signs such as nasal discharge, pulmonary congestion, diarrhea/dehydration and sluggishness. The incidence of these signs were transient and decreasing by the end of lactation.

Conclusions:

The following observations were made in this study where breeding dogs were given lufenuron at 3X the minimum recommended monthly use rate (10 mg/kg) on a *daily* basis (equivalent to 90X the monthly dose) prior to breeding, during mating, and through weaning (bitches): 1) fertility in the treated group was less than in the control group; 2) mean birth weight of pups born to treated bitches was less than that of pups born to control bitches; and 3) pups born to treated bitches showed an increased incidence of clinical signs compared to pups born to control bitches, though pup survival to weaning was greater in the treated group compared to the control group.

Additional studies were conducted to determine if these effects were related to treatment with lufenuron (see pivotal studies 7, 8, and 9).

Pivotal Study 7:

Controlled Trial Of Lufenuron In Reproducing Hounds

Type of Study:

The objective of this study was to evaluate the safety of lufenuron administered to reproducing large breed dogs.

Investigator/Study Director:

William R. Voss, D.V.M.
Hazleton Research Products, Inc.
Kalamazoo, MI

Animals:

Eighteen adult female and six adult male dogs of a mixed hound breed ranging in age from 1.5 to 5 years of age were used in this study. All but 3 of the dogs were proven breeders. The three maiden females were equally divided into the three treatment groups. The three study groups consisted of six females and two males each.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Placebo Tablets (swallow)

Dosage/Frequency of Treatment:

Lufenuron tablets were administered once every four weeks to one group at 1X and to a second group at 5X the minimum recommended monthly use rate of 10 mg/kg. The control group received placebo tablets equivalent to a 1X use rate. Females were initially dosed when first observed in proestrus and continued to be dosed until puppies were weaned. Males were initially dosed to coincide with the first female doses in their respective groups and continued to be dosed until all of their required females were bred. Study groups are described below:

	Number of		
Group	**Males**	**Females**	**Treatment**
1	2	6	1X Lufenuron
2	2	6	1X Placebo
3	2	6	5X Lufenuron

Two males were assigned to each group to allow for alternation of studs used for breeding and to provide enough studs in case of incompatibility between a bitch and stud.

Duration of Study: Seven Months

Evaluation:

Parameters assessed during this study consisted of physical examination, weekly observations for appearance and behavior, body weights, reproductive parameters to include fertility, whelping and weaning indices and gross necropsy of dead pups.

Results:

Results from the key reproductive parameters evaluated in this study are provided in the table below:

Group	**% Fertility**	**Mean No. Pups/Litter**	**% Pup Survival**
1X	83.3	9.6	86.6
Control	100	9.3	78.5
5X	100	7.1	97.2

Mean birth and weaning weights were comparable across all treatment groups.

Group (dose level)	**Mean Birth Weight (grams)**	**Mean Weaning Weight (grams)**
1X	423	3220
0X	388	2826
5X	467	2989

Some pups had to be fostered onto untreated females as a result of large litter sizes and/or inability of the female to sustain the litter. A total of 13 pups from control females, 15 pups from 1X females and 6 pups from 5X females were fostered. Because these pups did not nurse from study females for the entire lactation period, they were not included in the calculation of mean weaning weight. Two treated females, 1 in the 1X group and 1 in the 5X group had "possible" retained placentas and were treated with oxytocin. The diagnosis was not confirmed in these dogs. One treated female in the 1X group had a confirmed retained placenta. Physical examination and general observations made at whelping and weaning were similar between control and treated pups. Prior to weaning, 10 pups (from 2 litters) in the 5X group had mucopurulent nasal discharge. No gross necropsy findings considered

treatment-related were observed that differentiated between control and treatment pups.

Conclusions:

Fertility and mean birth weights in the 1X and 5X treatment groups were comparable to the concurrent controls, indicating that treatment with lufenuron had no adverse effect on these parameters. One treated female (1X group) had retained placenta and there was an increased incidence of mucopurulent nasal discharge in the pups born to 5X females.

REPRODUCTIVE SAFETY/CLINICAL STUDIES

Pivotal Study 8:

Controlled Clinical Trial of Lufenuron in Reproducing Small Breeds

Type of Study:

A controlled clinical study to evaluate the reproductive safety of lufenuron in small dog breeds under typical breeding kennel conditions.

Principal Investigator:

Larry Snyder, D.V.M.
Topeka, KS

Study Location:

Triple D Kennel
Berryton, KS

Dot's Little Doggies
Lyndon, KS

Animals:

A total of 127 dogs (99 female, 28 male), representing the following seven small breeds of dogs, were included in this study; Lhaso Apso, Miniature Dachshund, Miniature Pinscher, Miniature Schnauzer, Pomeranian, Shih Tsu and Yorkshire Terrier. Dogs selected were those routinely used for breeding at each site and only animals in good health and proven breeding ability were used. Animal study groups were formed following the usual practices and breeding plans at the two kennels. Each group consisted of two to five bitches and a single male, all of the same breed.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Placebo Tablets (swallow)

Dosage/Frequency of Treatment:

The following table describes the dosing groups used:

Group	Treatment Dose	Dosing Frequency
1	Lufenuron 1X	Every 4 Weeks
2	Placebo 1X	Every 4 Weeks
3	Lufenuron 5X	Every 2 Weeks
4	Placebo 5X	Every 2 Weeks

Dogs in the 1X treatment groups were dosed approximately every four weeks and dogs in the 5X treatment groups were dosed every two weeks. Males in all groups were dosed until their study group females were bred. Females in the 1X treatment groups were dosed until pups were weaned and females in the 5X group were dosed until whelping. A minimum of 2 and a maximum of 4 groups (each group consisting of 1 male and 2 to 5 bitches) were formed for each breed, depending on the number of dogs available. Each study group sire and all females in a group received the initial and all subsequent doses at the same time. In an attempt to have both treated and control groups represented for each breed, the following table was used to assign groups to specific treatments (i.e., if there were two groups of Yorkies available, 1 was randomly assigned to treatment with lufenuron and the other with the control, if 3 groups were available, 2 were randomly assigned to treatment with lufenuron and 1 to control, etc.):

Group/Breed	Study Group Assignment
2	1 Lufenuron Treated (1X) & 1 Control Group
3	2 Lufenuron Treated (1X and 5X) & 1 Control Group
4	2 Lufenuron Treated (1X and 5X) & 2 Control Groups

Blood Sampling: Blood samples were collected for lufenuron assay from males immediately prior to dosing on study days 14, 28, 56 and when released from the study. Blood samples were collected from females prior to dosing and on study days 14 and 28. Attempts were made to collect samples from females at estrus, early pregnancy and late pregnancy (blood samples could not be collected from all dogs at all specified time periods).

Evaluation:

Criteria evaluated in this study included the number of bitches producing litters in each study group, number of pups born and surviving for each litter, and pup birth weights.

Results:

Twenty-eight study groups representing seven breeds were evaluated. Four breeds were represented in all four treatment groups. Mean values, for the critical parameters evaluated and inclusive for all breeds are provided in the following table:

Group	Percent Whelping*	Percent Pup Survival	Number of Pups/Litter	Birth Weight (oz.)
1X Lufenuron	83	85	4.3	5.8
1X Placebo	83	67	4.0	5.8
5X Lufenuron	83	81	4.3	5.6
5X Placebo	77	74	5.4	5.9

*Percent whelping (litters produced/#bitches) was evaluated in this study (versus percent fertility) because individual animal observations were not possible when mating and pregnancy occurred. The major puppy mortality finding was stillborn pups, which occurred more in the control than treatment groups. Detailed clinical signs were not recorded for the puppies because such observations were not part of the normal procedures for the commercial kennels involved in this study.

Blood samples were collected at various study intervals to measure the lufenuron blood levels in the 5X group compared to the 1X group. For the males and females combined, the mean lufenuron level in group 1 was 0.796 ppm and in group 3 it was 2.279 ppm. This represents approximately a 2.9X difference. The males maintained higher blood levels than the females in this study. The group 3 males had an average of 3.8X higher blood levels than the group 1 males, while the group 3 females had an average of 2.7X higher blood levels than the group 1 females. **Conclusions:**

Lufenuron, administered to reproducing small breed dogs at 1X (monthly) and 5X (twice monthly) the minimum recommended monthly use rate of 10 mg/kg, did not have any adverse effects on the reproductive parameters measured in this controlled clinical study.

Pivotal Study 9:

Controlled Clinical Trial Of Lufenuron In Reproducing Large Breed Dogs

Type of Study:

A controlled clinical study to evaluate the reproductive safety of lufenuron administered to large breed dogs under typical breeding kennel conditions.

Principle Investigator:

Roger L. Sifferman, D.V.M.
Springfield, MO

Study Location:

Hanging Tree Kennel
Decatur, TX

Animals:

A total of 46 animals (38 female, 8 male) representing the following three large breeds of dogs were used in this study; English pointers, English Setters, and Walker

Hounds. The study took place at a commercial kennel. Housing conditions, routine handling, health care, maintenance, and male-female pairings were consistent with the kennel's usual practices. Only animals in good health were selected for enrollment in the study. Animals with prior histories of poor fertility, early fetal loss, abortion, peripartum infection, metritis and endometriosis were not used in the study. Four maiden females were used. Animal study groups consisted of one to fourteen bitches and a single male, all of the same breed.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Placebo Tablets (swallow)

Dosage/Frequency of Treatment:

Three study groups were formed comprising each of the three breeds. The following table describes the three groups evaluated in this study:

		No. Animals/Group	
Group	**Treatment**	**Male**	**Female**
1	Lufenuron 1X Monthly	3	12
2	Placebo 1X Monthly	3	12
3	Lufenuron 5X Monthly	2	14*

*One dog in group 3 was eliminated from the study after 1 month due to pre-existing renal disease.

Males were dosed until all respective study group females were bred. The 1X group females (control and treated) were dosed until pups were weaned. The 5X treatment group females were dosed until whelping.

Evaluation:

Criteria evaluated in this study included the number of bitches bred and pregnant, the number producing litters, the number of pups born and surviving per litter, pup birth weights, the number of pups per litter and clinical findings for dead pups.

Results:

Results from each of the critical parameters evaluated for the three study groups are provided in the table below:

Group	**Percent Fertility**	**Percent Pup Survival**	**Mean No. Pups/Litter**	**Mean Pup Birth Wt. (oz.)**
1X Lufenuron	83	88	8.0	14.5
1X Placebo	80	83	9.0	14.6
5X Lufenuron	80	87	9.5	14.9

Detailed clinical signs were not recorded for the puppies because such observations were not part of the normal procedures for the commercial kennels involved in this study. Post mortem evaluation of non-surviving pups did not reveal any treatment-related abnormalities.

Conclusion:

Lufenuron administered to reproducing large breed dogs at 1X and 5X the minimum recommended monthly dose of 10 mg/kg did not elicit any observed differences between lufenuron-treated and control groups.

Corroborative Studies

Study 1:

Acute Oral Toxicity Study (Tolerability) In Young Puppies With Lufenuron

Type of Study:

The objective of this study was to evaluate the acute oral toxicity of lufenuron to young puppies administered a single dose equivalent to 20X the monthly use rate.

Investigator/Study Director:

Dr. Edwin Goldenthal
International Research and Development Corp.
Mattawan, MI

Animals:

Six male and six female purebred beagle dogs, eight weeks of age, were used in this study. The two study groups contained 3 male and 3 female dogs each. The dogs were housed in groups of 3 by treatment group and sex.

Dosage Form: Lufenuron Tablets (swallow)

Controls: Untreated

Dosage/Frequency of Treatment:

Lufenuron was administered in a single dose by tablet combinations to achieve 200 mg/kg (20X the recommended dose). A control group was left untreated.

Study Duration: Fifteen days post treatment.

Evaluation:

Mortality, moribundity, body weights, clinical signs, hematological and biochemical parameters were the criteria evaluated for treatment effects.

Results:

A lufenuron-treated male puppy died on day 5 with clinical findings of decreased activity, unidentified red material in the feces and body tremors. The major necropsy findings were edematous kidneys with thickened capsules and cystitis. The other treated puppies exhibited decreased appetite during days 8-11 and the surviving two treated males exhibited decreased activity on days 10-11. Red colored material was observed in the feces of the two surviving treated males during study days 10-13. Incidental clinical signs, including diarrhea and lacrimation, were observed in several animals in both the control and treated groups. There were no marked changes or differences observed in body weight, hematological or biochemical parameters.

Conclusion:

Lufenuron, when administered as a single dose equivalent to 20X the recommended use rate, may cause decreased activity and appetite in eight week old puppies. The mean body weights were higher in the treated versus control group at the end of the study.

Study 2:

14-Day Probe Study with Lufenuron in Male Beagle Dogs .

Investigator/Study Director: S.M. MacAskill, K. L. Pavkov

Study Location:

Ciba-Geigy Corporation
Environmental Health Center
Farmington, CT

Animals: Two 11-month-old male beagle dogs.

Dosage/Frequency of Treatment:

Each of the two dogs received one 45 mg/kg dose of lufenuron (4.5X) administered in an oral 15% suspension into the back of the mouth.

Controls: None

Study Duration: 14 Days

Results:

The single 45 mg/kg dose produced no signs of toxicity measured by clinical signs, hematological, clinical chemistry, food consumption and organ weight parameters.

Study 3:

28-Day Range-Finding Study with Lufenuron in Beagle Dogs.

Investigator/Study Director: K. L. Pavkov, S. M. MacAskill

Study Location:

Ciba-Geigy Corporation
Environmental Health Center
Farmington, CT

Animals:

Two 8-month-old male beagle dogs, two 8-month-old female beagle dogs.

Dosage/Frequency of Treatment:

Four dogs were dosed daily for 28 days, with an oral 15% suspension of lufenuron at 50 mg/kg (5.0X).

Controls: None

Study Duration: Twenty-eight days.

Results:

All four dogs tolerated lufenuron without overt or covert signs of toxicity at 50 mg/kg body weight given daily for 28 days. Clinical signs, mortality, ophthalmoscopic examination, body weights and food consumption, clinical chemistry and organ weights were parameters measured.

Study 4:

A Pregnant Bitch Study with Lufenuron.

Investigator/Study Director: Dr. Martin R. Gilman

Study Location:

Hazleton-LRE
Kalamazoo, MI

Animals: Five proven beagle bitches.

Dosage/Frequency of Treatment:

Beginning with the first mating, five proven female beagle bitches were dosed daily at 30 mg/kg with an orally administered 15% suspension of lufenuron until 21 days post-whelping.

Controls: None

Results:

Animals were observed daily and body weights and puppy health parameters were evaluated. Three of the five bitches bred conceived. Pregnant bitches tolerated lufenuron and produced normal progeny. Mean puppy birth weight was 0.23 kg. All surviving puppies appeared thrifty. Only 2 puppies died, both deaths thought to be unrelated to treatment.

Study 5:

Pregnant Bitch Toxicity Study in Beagles .

Investigator/Study Director: Dr. Martin R. Gilman

Study Location:

Hazleton-LRE
Kalamazoo, MI

Animals:

Ten proven beagle bitches were randomly placed into two groups of five animals each.

Dosage/Frequency of Treatment:

Group 1 received 30 mg/kg lufenuron daily beginning with the first tie, continuing through gestation and for twenty-one days post whelping. Group 2 received the same dosage for the same duration but were treated once per week.

Dosage Form: Tablets (swallow)

Controls: None

Results:

Nine of the ten bitches on test conceived, one exhibited pseudo-pregnancy. Both pregnant bitches and their progeny tolerated lufenuron administered at 30 mg/kg weekly or daily to the bitches. The mean birth weight was 0.25 kg.

The average lufenuron blood concentration in group 1 bitches was approximately 10X that of group 2. Blood concentrations in 6-7 week old puppies whelped and nursing from group 1 bitches were 7X those whelped from group 2 bitches. Blood concentrations in pups drawn at the same time as their respective dams were 4-6X higher in the puppies compared to the dams. Elevated lufenuron levels were detectable in puppies 4-5 weeks after weaning.

Comparison of blood and milk samples collected indicates that lufenuron concentrates in the milk of treated bitches. Milk concentrations were from 14-49X higher than blood concentrations in bitches when measured 49-56 days post whelping.

Study 6:

Overt Toxicity Study in Dogs with Lufenuron Evaluating Males, Non-Pregnant Females, Pregnant Females and Their Puppies.

Investigator/Study Director: Dr. Martin R. Gilman

Study Location:

Hazleton-LRE
Kalamazoo, MI

Animals:

Ten male and twenty female beagle dogs, 10-22 months of age, were placed into six groups (5 per group) - two groups of unbred females, two groups of bred females, two groups of males not used for breeding.

Dosage/Frequency of Treatment:

One group each of unbred and bred females and unbred males received either 20 (2X recommended dose) or 60 (6X recommended dose) mg/kg lufenuron. Bred females were dosed after mating and once every four weeks through gestation and through day 28 post-whelping. Unbred females were dosed on the same schedule. Males were treated once and observed for 30 days.

Dosage Form: Lufenuron Tablets (swallow)

Results:

All 10 females bred become pregnant. The mean birth weight of pups born to females treated at 2X was 227.5 grams compared to 290.7 grams for the pups born to females treated at 6X. No overt toxicity was observed in any of the parameters evaluated including clinical observations, daily observations, body weights, pup survival and gross necropsy of any dead pups.

Study 7:

Pharmacokinetics and Dose Proportionality of Lufenuron in Male Beagles, Pregnant vs. Non-Pregnant Beagles and Beagle Pups.

Investigator/Study Director: Dr. Janis MacKichan

Study Location:

Ohio State University
Columbus, Ohio

Results:

This study involved the analysis of lufenuron in blood and milk samples collected from the previous study (Corroborative Study 6) conducted at Hazleton-LRE.

The average milk:blood concentration ratio was approximately 60 (i.e. 60X higher concentration in the milk compared to the levels in the blood of treated bitches). Blood concentrations were higher in pregnant bitches during the gestation period as compared to the nonpregnant bitches, but lower during the lactation period. The lower blood concentrations in lactating bitches as compared to nonlactating bitches was attributed to the significant excretion of lufenuron in milk. Pertinent blood/milk information is provided below:

Group	**# Bitches Per Group**	**Avg Blood Conc. In Bitches (ng/mL)**	**Avg Milk Conc. In Bitches (ng/mL)**	**Avg Blood Conc. In Litters (ng/mL)**
1 (20 mg/kg)	5	58.2	3293.2	458
2 (60 mg/kg)	5	140.4	6645.8	1309

Conclusions: Lufenuron concentrates in the milk of treated bitches and will be passed to nursing pups throughout the lactation period. Pups nursing off their treated dams may have approximately 8-9 times higher concentrations of lufenuron in their blood compared to the dam.

TABLE 3 PIVOTAL TARGET ANIMAL SAFETY STUDIES

Study No./Type	**Study Director/Investigator**	**Study Location**
1) Ten-Month Oral Toxicity	Dr. Edwin Goldenthal	IRDC* Mattawan, MI
2) Six-Month Oral Toxicity In Combination With Insecticides	Dr. Edwin Goldenthal	IRDC Mattawan, MI
3) Six-Week Oral Toxicity In Combination With Chlorpyriphos	Dr. Edwin Goldenthal	IRDC Mattawan, MI
4) Acute Oral Toxicity (Tolerability) Study In Dogs	Dr. Edwin Goldenthal	IRDC Mattawan, MI
5) 90-Day Toxicity Study In Young Beagle Dogs (6-Weeks or Less in Age)	Dr. Martin Gilman	Hazleton, Inc. Kalamazoo, MI
6) A Reproduction Study In Beagle Dogs	Mr. James Schardein	IRDC Mattawan, MI
7) Controlled Clinical Trial In Reproducing Hounds	Dr. William R. Voss	Hazleton, Inc. Kalamazoo, MI
8) Controlled Clinical Trial In Reproducing Small Breeds	Dr. Larry Snyder	Berryton and Lyndon, KS
9) Controlled Clinical Trial In Reproducing Large Breed Dogs	Dr. Roger L. Sifferman	Decatur, TX

*International Research and Development Corporation

IV. HUMAN FOOD SAFETY

Data on human safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. This drug is to be labeled for use in dogs which are non-food animals.

V. AGENCY CONCLUSIONS

The data in support of this NADA comply with the requirements of Section 512 of the Act and Section 514.111 of the implementing regulations. It demonstrates that PROGRAM® Tablets (lufenuron), when used under labeled conditions of use, are safe and effective.

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is judged to be critical in understanding that this drug works to prevent and control flea populations by inhibiting flea egg development. If the product is used without knowledge of the flea's life cycle and without the knowledge of which topical flea adulticides are required and when they should be used, the drug will not work effectively.

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 (including any ester or salt of the active ingredient) has been approved in any other application.

VI. LABELING (ATTACHED)

A. Package Inserts
   - Veterinarian's Insert
   - Owner's Insert

B. Blister Packs--45 mg, 90 mg, 204.9 mg, and 409.8 mg
C. Display Cartons--45 mg, 90 mg, 204.9 mg, and 409.8 mg
D. Shipping Cartons--45 mg, 90 mg, 204.9 mg, and 409.8 mg

Copies of these labels may be obtained by writing to the:

Freedom of Information Office
Center for Veterinary Medicine, FDA
7500 Standish Place
Rockville, MD 20855

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.