

## EFFICACY REVIEW

**PRODUCT:** CERTIFECT

**DATE:** November 3, 2010

**FILE SYMBOL:** 65331-T

**DP BARCODE:** 372045

**DECISION:** 423378

**GLP:** No

**CHEMICALS:** Fipronil (9.8%) + (S)-Methoprene (8.8%) + Amitraz (22.1%)

**CHEMICAL NUMBERS:** Fipronil - 129121 (S)-Methoprene - 105402 Amitraz - 106201

**PURPOSE:** To determine efficacy for registration of new spot-on combination product for dogs.

### **MRIDs:**

*47914207. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Dermacentor variabilis on Dogs.*

*47914208. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Dermacentor reticulatus on Dogs.*

*47914209. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Ixodes scapularis on Dogs.*

*47914210. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Ixodes ricinus on Dogs.*

*47914211. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Amblyomma americanum on Dogs.*

*47914212. A Study to Select the Dose of ML-3,948,906 to be combined with ML-2,095, 988 509T for a Single Topical Treatment on Dogs.*

*47914213. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Amblyomma maculatum on Dogs.*

*47914214. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Haemaphysalis elliptica on Dogs.*


*47914215. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Dog Fleas (Ctenocephalides canis) on Dogs.*

*47914216. A Study to Evaluate the Ability of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 to Prevent the Transmission of Babesia canis (Piana & Galli-Valerio, 1895) from Infected Dermacentor reticulatus (Fabricius, 1794) to Dogs.*

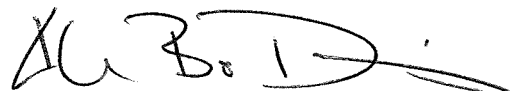
*47914217. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Haemaphysalis longicornis on Dogs.*

- 47914218.** *Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Ixodes holocyclus on Dogs.*
- 47914219.** *Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Amblyomma maculatum on Dogs.*
- 479141220.** *A Study to Evaluate the Ability of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 to Prevent the Transmission of Borrelia burgdorferi from Infected Ixodes scapularis to Dogs.*
- 47914221.** *Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Ixodes scapularis on Dogs that Undergo Weekly Water Immersion or Single Shampooing Post Treatment.*
- 47914222.** *Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Rhipicephalus sanguineus on Dogs that Undergo Single Shampooing Post Treatment*
- 47914223.** *A Study to Evaluate the Prevention of Attachment Effects of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Rhipicephalus sanguineus & Dermacentor variabilis ticks on Dogs.*
- 47914224.** *A Study to Determine the Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 to Cause a Detachment of Ticks.*
- 47914225.** *A Study to Evaluate the Prevention of Attachment Effects of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Rhipicephalus sanguineus & Dermacentor variabilis ticks on Dogs.*
- 47914226.** *Effectiveness of Two Topical Treatments one Month Apart with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Rhipicephalus sanguineus on Dogs.*
- 47914227.** *Combination Testing of Amitraz and Fipronil in the Rhipicephalus sanguineus Contact Test.*
- 47914228.** *The Motility of Ticks Exposed to Amitraz and Fipronil Residues on a Petri Dish Using the Lemna Tec Scanalyzer Imaging System.*

**TEAM REVIEWER:** Autumn Metzger

**EFFICACY REVIEWER:** Autumn Metzger, M.S.  11/3/10

**SECONDARY EFFICACY REVIEWER:** Kable Bo Davis, M.S.

 11-3-10

**BACKGROUND**

CERTIFECT is a topical spot-on insecticide treatment for the control of fleas and ticks for dogs and puppies 7 weeks of age and older. The product is applied on the skin between the shoulder blades once monthly. This product is similar to the already registered product, Frontline Plus with the addition of the chemical amitraz for added tick efficacy.

Labeled Application Rates:

Dog Size	Application Rate
Dogs & Puppies older than 7 weeks and up to 22 lbs	.036 fl oz
Dogs 23-44 lbs	.072 fl oz
Dogs 45-88 lbs	.145 fl oz
Dogs 89-132 lbs	.217 fl oz

**DATA REVIEW**

The following data review is comprised of explanations of materials and methods, and a summation of experimental results containing tables with reformatted data.

**MRID 47914207. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Dermacentor variabilis* on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination product ML-2,095, 988 509T and ML-3,948,906 (frontline plus + amitraz) against the “American Dog Tick.”

**Set Up**

Sixteen beagle dogs aged 9-24 months old were split into 2 groups of 8 dogs. Groups 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28 & 35 each dog was infested with 50±5 ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 18, 24 & 48 hour counts up to day 30.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:

(\*) indicates statistically significant over non treated control

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	62.4*	1	86.8*	2	99.5*
7	99.3*	8	99.7*	9	100*
14	97.1*	15	99.7*	16	100*
21	96.5*	22	98.4*	23	99.8*
28	63.9*	29	83.2*	30	98.5*
35	<0	36	<0	37	64.8*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls *Dermacentor variabilis* ticks.

**MRID: 47914208. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Dermacentor reticulatus* on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the “Marsh tick.”

**Set Up**

Sixteen beagle dogs aged 6-10 months old were split into 2 groups of 8 dogs. Groups 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35, 42 & 49 each dog was infested with 50±2 ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 18, 24 & 48 hour counts except 18 hours, day zero.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:

(\*) indicates statistically significant over non treated control

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	49.5	1	82.3*	2	100*
7	100*	8	100*	9	100*
14	95.6*	15	98.4*	16	100*
21	94.7*	22	96.9*	23	100*
28	87.9*	29	94.5*	30	98.9*
35	63.6*	36	73.3*	37	93.8*
42	42.2*	43	59.2*	44	94.3*
49	29.9*	50	34.9*	51	84.2*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls *Dermacentor reticulatus* ticks.

**MRID 47914209. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Ixodes scapularis* on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the “Black legged or deer tick.”

### Set Up

Sixteen beagle dogs aged 6 months - 6 years old were split into 2 groups of 8 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with 50 ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

### Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each 18, 24 & 48 hour tick count.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:

(\*) indicates statistically significant over non treated control

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	91.7*	1	98.2*	2	99.3*
7	100*	8	100*	9	100*
14	100*	15	100*	16	100*
21	99.4*	22	100*	23	100*
28	100*	29	98.8*	30	99.5*
35	98.3*	36	98.2*	37	100*
42	97.7*	43	97.2*	44	99.7*

### Conclusion

The study supports label claims that the combination product prevents, kills and controls *Ixodes scapularis* ticks.

**MRID 47914210. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Ixodes ricinus* on Dogs.**

### Objective

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the "Castor bean tick."

### Set Up

Sixteen beagle dogs aged 12 months - 6 years old were split into 2 groups of 8 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with 50  $\pm$  2 ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

### Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each 18, 24 & 48 hour tick count.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:  
 (\*) indicates statistically significant over non treated control

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	49.7*	1	78*	2	97.9*
7	98.9*	8	99.7*	9	100*
14	99*	15	99.4*	16	100*
21	97.5*	22	98.2*	23	98.1*
28	89.9*	29	93.4*	30	97.7*
35	73.7*	36	82.7*	37	96.3*
		43	57.2*	44	80.1*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls *Ixodes ricinus* ticks.

**MRID 47914211. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Amblyomma americanum* on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the “lone star tick.”

**Set Up**

Sixteen beagle dogs approximately 3 years old were split into 2 groups of 8 dogs. Groups 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with 50 ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at some of the 18, 24 & 48 hour tick counts.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:  
 (\*) indicates statistically significant.

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	<0	1	56.9*	2	95.8*
7	100 *	8	100*	9	100*
14	100*	15	100*	16	100*
21	95.6*	22	97.2*	23	100*
28	70.1	29	69.7	30	95.3*
35	62.3	36	76.1	37	91.8*
42	53.2*	43	70.2	44	79.3

## Conclusion

The study supports label claims that the combination product prevents, kills and controls *Amblyomma americanum* ticks.

## MRID 47914212. A Study to Select the Dose of ML-3,948,906 to be combined with ML-2,095, 988 509T for a Single Topical Treatment on Dogs.

## Objective

The trial objective was to determine the dose of amitraz that would provide superior efficacy when combined with the product Frontline Plus (fipronil + (S)-methoprene) against *Rhipicephalus sanguineus*, commonly known as the brown dog tick.

## Set Up

Thirty beagle dogs approximately 3 – 6 years old were used. Dogs were broken into 6 groups of 5 dogs and treated as follows:

Group 1 – untreated control

Group 2- Frontline Top Spot (fipronil only product)

Group 3- Frontline Plus (fipronil + (S)-methoprene) +  $\geq 4$  mg/kg of amitraz

Group 4 – Frontline Plus (fipronil + (S)-methoprene) +  $\geq 8$  mg/kg of amitraz

Group 5 - Frontline Plus (fipronil + (S)-methoprene) +  $\geq 16$  mg/kg of amitraz

All dogs were infested on days -1, 7, 14, 21, 28, 35 & 42 with 50  $\pm$ 5 ticks. Ticks were counted at 24 & 48 hours post infestation then removed at 48 hours.

## Results

Efficacy Rates Based on Geometric means of tick counts at 24 and 48 hours:

(\*) indicates statistically significant over non treated control

Group 2 vs. Untreated control			
24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy
1	55.2*	2	86.9*
8	95.1*	9	99.5*
15	86.5*	16	97.5*
22	44.4*	23	89.6*
29	64.3*	30	88.0*
36	49.0*	37	46.1
43	32	44	28.2

Group 3 vs. Untreated control			
24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy
1	49.3*	2	92.8*
8	98.4*	9	99.1*
15	100*	16	100*
22	93.7*	23	98.0*
29	86.6*	30	98.9*
36	65.6*	37	76.8*
43	62.0*	44	74.8*

Group 4 vs. Untreated control			
24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy
1	77.2*	2	99.3*
8	100*	9	99.5*
15	100*	16	100*
22	100*	23	100*
29	99.3*	30	100*
36	0.9*	37	96.0*
43	88.6*	44	92.0*

Group 5 vs. Untreated control			
24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy
1	79.7*	2	96.7*
8	100*	9	100*
15	100*	16	100*
22	97.1*	23	98.7*
29	97.0*	30	99.3*
36	93.3*	37	99.9*
43	89.2*	44	91.1*

Dogs treated with  $\geq 4\text{mg/kg bw}$  (group 3) had significantly greater ( $p \leq .05$ ) tick counts vs. Frontline Top Spot on days 15 & 22 at 24 hours and days 23, 30 & 44 at 48 hours.

Dogs treated with  $\geq 8\text{mg/kg bw}$  (group 4) had significantly greater ( $p \leq .05$ ) tick counts vs. Frontline Top Spot at 24 hours on days 15, 22, 29, 36 & 43 and at 48 hours on days 2, 23, 30, 37 & 44.

Dogs treated with  $\geq 16\text{mg/kg bw}$  (group 5) had significantly greater ( $p \leq .05$ ) tick counts vs. Frontline Top Spot at 24 hours on days 15, 36 & 43 and on days 2, 30, 37 & 44 at 48 hours.

### Conclusion

They chose the dose  $\geq 8\text{mg/kg bw}$  of amitraz for the combination of Frontline Plus since it performed significantly better at the 24 hour tick count than Frontline Top Spot (group 2) alone and group 3, but was not significantly different than group 5.

### MRID 47914213. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Amblyomma maculatum* on Dogs.

### Objective

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the "Gulf coast tick."

### Set Up

Sixteen beagle dogs approximately 1-10 years old were split into 2 groups of 8 dogs. Groups 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with  $30 \pm 5$  ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.



## Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 18, 24 & 48 hour tick counts.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:

(\*) indicates statistically significant.

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	87.4*	1	95.0*	2	99.5*
7	100*	8	100*	9	100*
14	100*	15	100*	16	100*
21	96.5*	22	100*	23	100*
28	89.0*	29	95.3*	30	100*
35	83.9*	36	92.8*	37	97.1*
42	66.9*	43	70.2*	44	84.2*

## Conclusion

The study supports label claims that the combination product prevents, kills and controls *Amblyomma maculatum* ticks.

## MRID 47914214. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Haemaphysalis elliptica* on Dogs.

## Objective

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the "African Yellow Dog Tick."

## Set Up

Sixteen beagle or mongrel dogs > 52 weeks were split into 2 groups of 8 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with  $50 \pm 5$  ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

## Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 18, 24 & 48 hour tick counts.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:

(\*) indicates statistically significant.

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	57.3*	1	84.2*	2	99.3*
7	100*	8	100*	9	100*
14	98.6*	15	100*	16	100*
21	97.1*	22	98.3*	23	99.6*
28	93.1*	29	92.5*	30	98.1*
35	82.6*	36	92.8*	37	95.9*
42	83.3*	43	87.5*	44	88.8*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls *Haemaphysalis elliptica* ticks.

**MRID 47914215. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of Dog Fleas (*Ctenocephalides canis*) on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against fleas.

**Set Up**

Sixteen beagle > 3 years were split into 2 groups of 8 dogs. Groups 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35, 42, 49 & 56 each dog was infested with 100 ±2 fleas. Fleas were counted then removed at 24 hours.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of fleas than untreated controls at each of the counts.

Efficacy Rates Based on Geometric means of flea counts at 24 hours:

(\*) indicates statistically significant.

24 hours	
Day	% Efficacy
1	99.9*
8	100*
15	100*
22	100*
29	99.8*
36	99.6*
43	99.3*
50	96.5*
57	73.0*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls dog flea infestations up to 50 days, or 7 weeks.

**MRID 47914216. A Study to Evaluate the Ability of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 to Prevent the Transmission of *Babesia canis* (Piana & Galli-Valerio, 1895) from Infected *Dermacentor reticulatus* (Fabricius, 1794) to Dogs.**

Experiments were conducted to determine if the combination product could prevent transmission of *Babesia canis* to dogs. It should be noted that the Agency does not permit disease claims on labels and therefore this study was not reviewed.

**MRID 47914217. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Haemaphysalis longicornis* on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the “bush tick.”

**Set Up**

Sixteen beagle or mongrel dogs 24-46 weeks were split into 2 groups of 8 dogs. Groups 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with 50 ticks. Ticks were counted at 18, 24 & 48 hours post infestation then removed at 48 hours.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 18, 24 & 48 hour tick counts.

Efficacy Rates Based on Geometric means of tick counts at 18, 24 and 48 hours:

(\*) indicates statistically significant.

18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	80.3*	1	100*	2	100*
7	100*	8	100*	9	100*
14	100*	15	100*	16	100*
21	100*	22	100*	23	100*
28	100*	29	100*	30	100*
35	97.6*	36	99.6*	37	100*
42	91.6*	43	97.6*	44	100*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls *Haemaphysalis longicornis* ticks.

**MRID 47914218. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Ixodes holocyclus* on Dogs.**

**Objective**

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the “paralysis tick.”

### Set Up

Sixteen foxhound dogs approximately 1.6 to 7.6 years old were split into 2 groups of 8 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28 & 35 each dog was infested with 30 ticks. Ticks were counted at 24, 48 & 72 hours (days 1, 2 & 3) post infestation then removed 72 hours after each infestation.

All dogs were hyper-immunized against the *I. holocyclus* toxin.

### Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls some of the 24, 48 & 72 hour tick counts.

Efficacy Rates Based on Geometric means of tick counts at 24, 48 and 72 hours:

(\*) indicates statistically significant.

24 hours		48 hours		72 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
1	56.3*	2	86.9*	3	99.6*
8	98*	9	99.1*	10	100*
15	94.5*	16	97.3*	17	99.6*
22	73*	23	85.6*	24	95.5*
29	61.2*	30	75*	31	96.9*
36	25.1	37	39.9*	38	53.4*

### Conclusion

The study supports label claims that the combination product prevents, kills and controls *Ixodes holocyclus* ticks.

**MRID 47914219. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Amblyomma maculatum* on Dogs.**

### Objective

The trial objective was to determine the 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) against the "Gulf coast tick."

### Set Up

Sixteen beagle or mongrel dogs 1-11 years were split into 2 groups of 8 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero. On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with 30 ticks. Ticks were counted at 6, 12, 24 & 48 hours post infestation then removed at 48 hours.

### Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 6, 12, 24 & 48 hour tick counts.

Efficacy Rates Based on Geometric means of tick counts at 6, 12, 24 & 48 hours:  
 (\*) indicates statistically significant.

6 hours		12 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	72.9*	0	84.7*	1	97.1*	2	99.3*
7	98.7*	7	98.7*	8	100*	9	100*
14	99.0*	14	99.4*	15	100*	16	100*
21	81.5*	21	88.8*	22	93.8*	23	97.8*
28	46.9*	28	71.3*	29	89.4*	30	95.2*
35	81.8*	35	87.2*	36	89.4*	37	93.5*
42	43.7*	42	74.4*	43	86.8*	44	92.1*

**Conclusion**

The study supports label claims that the combination product prevents, kills and controls *Amblyomma maculatum* ticks starting after 12 hours of exposure.

**MRID 479141220. A Study to Evaluate the Ability of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 to Prevent the Transmission of *Borrelia burgdorferi* from Infected *Ixodes scapularis* to Dogs.**

Experiments were conducted to determine if the combination product could prevent transmission of *Borrelia burgdorferi* to dogs. It should be noted that the Agency does not permit disease claims on labels and therefore this study was not reviewed.

**MRID 47914221. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Ixodes scapularis* on Dogs that Undergo Weekly Water Immersion or Single Shampooing Post Treatment.**

**Objective**

The trial objective was to determine if 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) after water immersion or shampooing.

**Set Up**

Twenty-four beagle dogs 4-8 years were split into 3 groups of 8 dogs.

Group 1 = was a non-treated control

Group 2 & 3 = were treated with the combination product on day zero.

Groups 1 & 2 were water immersed on days 10, 24, 31 & 38

Group 1 & 3 were shampooed with Allergroom shampoo on day 17

Group 2 was water immersed only on day 17

**Definitions (for the purpose of their study):**

**Water Immersion** = submersed in a barrel or tub of water (head above) for at least 1 minute. The head of each dog was wetted 3 x during submersion.

**Shampooing** = Dogs were wetted and lathered on dorsal midline, sides, rear, chest & ventrum. Dogs face was washed carefully. Lather was left on for 5 minutes then dogs were thoroughly rinsed.

On days -1, 7, 14, 21, 28, 35 & 42 each dog was infested with 50 ± 5 ticks. Ticks were counted and removed 48 hours after each infestation.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 48 hour tick counts.

Efficacy Rates as Compared to Non-treated Control Based on Geometric means of tick counts at 48 hours:

(\*) indicates statistically significant.

Group 1 (control)		Group 2		Group 3	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
2	22.4	2	99.7*	2	96.6*
9	23.7	9	99.6*	9	100*
16	25.4	16	100*	16	100*
23	6.4	23	100*	23	100*
30	14.0	30	100*	30	100*
37	15.0	37	100*	37	100*
44	14.2	44	100*	44	100*

**Conclusion**

The study supports label claims that the combination product is water proof and that it is still effective after shampooing, bathing or swimming.

**MRID 47914222. Effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Rhipicephalus sanguineus* on Dogs that Undergo Single Shampooing Post Treatment.**

**Objective**

The trial objective was to determine if 1 dose of the combination of ML-2,095, 988 509T and ML-3,948,906 (which is frontline plus + amitraz) after a single shampooing.

**Set Up**

Sixteen beagle dogs 14 months to 6 years were split into 2 groups of 8 dogs.

Group 1 = was a non-treated control

Group 2 = were treated with the combination product on day zero.

Each dog was infested with  $50 \pm 5$  ticks on days -1, 7, 14, 21, 28, 35 & 42. Ticks were counted and removed 48 hours after each infestation. All dogs were shampooed with Bio Guard on day 17.

Refer to 47914221 for shampoo description.

### Results

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at each of the 48 hour tick counts.

Efficacy Rates as Compared to Non-treated Control Based on Geometric means of tick counts at 48 hours:

(\*) indicates statistically significant.

48 hours	
Day	% Efficacy
2	90.3*
9	100*
16	100*
23	98.9*
30	93.9*
37	91.1*
44	76.5*

### Conclusion

The study supports label claims that the combination product is water proof and that it is still effective after shampooing, bathing or swimming.

**MRID 47914223. A Study to Evaluate the Prevention of Attachment Effects of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against *Rhipicephalus sanguineus* & *Dermacentor variabilis* ticks on Dogs.**

### Objective

The primary objective was to determine the percent reduction in attached ticks using the combination product vs. an untreated control. The secondary objective was to determine mortality of ticks that had not attached.

### Set Up

Twenty beagle dogs 4-11 years were split into 2 groups of 10 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero.

Dogs were exposed to  $50 \pm 5$  *R. sanguineus* ticks on days 1 & 7 and  $50 \pm 5$  *D. variabilis* on days 1, 7, 14, 21 & 28 by placing the ticks on the bottom of a plastic crate then placing the dogs in the crate. (Low infestation rates of the *R. sanguineus* ticks on the control group resulted in a discontinuation of this species use after day 7.)

Dogs were moved to another plastic crate ~2 hours after infestation and the ticks remaining in the first crate were counted (live & dead). After 4 hours in the second crate, dogs were removed and ticks remaining in the crate were counted (live & dead). Live ticks from both crates were placed in vials for 24 hours and any dead ones after this time period were recorded. Twenty-four hours after the dogs were removed from the crates, remaining ticks on the dogs were counted and removed.

**Results**

Primary (attachment) –

For the *R. sanguineus* ticks: dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at 4 hours on days 1 & 7 (98.0%, 98.0%) and at 24 hours on days 2 & 8 (98.7%, 100%) but was discounted after that due to low tick attachment in the control.

For the *D. variabilis* ticks: dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls at 4 hours on days 1 & 7 and at 24 hours on days 2, 8, 15, 22 & 29.

Efficacy Rates Based on Geometric means of tick counts vs. untreated control at 4 & 24 hours: (\*) indicates statistically significant.

4 hours		24 hours	
Day	% Efficacy	Day	% Efficacy
1	96.0*	2	100*
7	92.2*	8	99.7*
14	10.6	15	100*
21	22.3	22	98.3*
28	-15.6	29	87.4*

**Primary Conclusion (attachment)**

The study showed significantly lower attachment at 4 hours on days 1 & 7 only. On day 28, there were actually more ticks on treated dogs than on the control. On days 15 & 22 a reduction of 10.6% & 22.3% was not significant. This study does not support label claims that the combination product prevents attachment.



## Results

### Secondary (% mortality of ticks collected in crates after 24 hours) –

Efficacy Rates Based on Geometric means of tick counts vs. untreated control at 2, 4 & 24 hours:

(\*) indicates statistically significant.

2 hours		4 hours		24 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
<b><i>R. Sanguineus</i></b>					
1	59.3*	1	70.1*	2	100*
7	53.3*	7	76.4*	8	100*
<b><i>D. Variabilis</i></b>					
1	20.3*	1	27.3*	2	97.1*
7	14.1*	7	45.4*	8	100*
14	4.6*	14	10.3*	15	98.3*
21	-0.6	21	4.3	22	58.6*
28	0.4	28	2.0*	29	33.3

### Secondary Conclusion (% mortality of ticks collected in crates)

After 24 hours of exposure, there were cumulative % mortalities of treated ticks >80%. Therefore it can be concluded exposure alone to the combo product can kill ticks within 24 hours of exposure without ticks having to become attached. This does not support any “quick” or “quickly kills” claims on the label, nor does it prove that ticks didn’t or couldn’t attach.

### **MRID 47914224. A study to determine the effectiveness of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 to Cause a Detachment of Ticks.**

#### **Objective**

To determine if the combination product will cause detachment of ticks.

#### **Set Up**

Sixteen beagle dogs 5-7 years were split into 2 groups of 8 dogs. Group 1 was a non-treated control and group 2 was treated with the combination product on day zero. Each dog was infested with  $50 \pm 5$  *R. Sanguineus* and  $50 \pm 5$  *D. Variabilis* ticks on day -2.

After 24 hours (day -1) ticks were counted and attachment locations with species of tick were mapped out for each dog; then again at 4, 12 & 24 hours after treatment (day 0). All ticks were counted, mapped then removed at 48 hours after treatment.

## Results

Efficacy Rates Based on Geometric means of tick counts and % detachment at each time period (\*) indicates statistically significant.

### *R. sanguineus*

TIME	Geo. Mean of control	Geo. Mean of combo	% detachment
Day -1	27.2	22.9	na
Day 0, 4 hrs	17.5	12.2	30.3*
Day 0, 12 hrs	19.5	3.4	82.8*
Day 1, 24 hrs	19.5	0.3	98.7*
Day 2, 48 hrs	17	0.2	98.9*

### *D. variabilis*

TIME	Geo. Mean of control	Geo. Mean of combo	% detachment
Day -1	30.4	29	na
Day 0, 4 hrs	22.7	19.5	14.5
Day 0, 12 hrs	26.5	4.3	83.9*
Day 1, 24 hrs	26.1	1.9	92.8*
Day 2, 48 hrs	27.1	0.3	98.9*

## Conclusion

The study supports label claims that the combination may cause tick detachment starting after 12 hours of exposure. It does not support any “quick” or “quickly” claims

**MRID 47914225. A Study to Evaluate the Prevention of Attachment Effects of a Single Topical Treatment with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against *Rhipicephalus sanguineus* & *Dermacentor variabilis* ticks on Dogs.**

## Objective

This study is identical to 47914223 with the exception that both species of ticks were tested on days 1, 7, 14, 21 & 28. The primary objective was to determine the percent reduction in attached ticks using the combination product vs. an untreated control. The secondary objective was to determine mortality of ticks that had not attached.

## Set Up

Please refer to 47914223.

## Results

Primary (attachment) –

Efficacy Rates Based on Geometric means of tick counts vs. untreated control at 4 & 24 hours:  
(\* ) indicates statistically significant.

4 hours		24 hours	
Day	% Efficacy	Day	% Efficacy
1	93.8*	2	99.3*
7	92.5*	8	99.2*
14	67.1	15	97.6*
21	51.3*	22	97.1*
28	37.2	29	94.5*

### Primary Conclusion (attachment)

For the purposes of labeling, we can conclude that the combination product resulted in a reduction in attachment >80% at 4 hours on days 1 & 7 and can only be labeled with that qualification.

## Results

Secondary (% mortality of ticks collected in crates after 24 hours) –

Efficacy Rates Based on Geometric means of tick counts vs. untreated control at 2, 4 & 24 hours:  
(\* ) indicates statistically significant.

2 hours		4 hours		24 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
<b><i>R. Sanguineus</i></b>					
1	22.9*	1	37.2*	2	na
7	25.5*	7	41.1*	8	na
14	9.2*	14	32.2*	15	94.3*
21	3.4*	21	10.8*	22	96.9*
28	7.8*	28	11.0*	29	7.0*
<b><i>D. Variabilis</i></b>					
1	14.2*	1	27.5*	2	na
7	20.9*	7	34.6*	8	na
14	1.6	14	11.2*	15	71.1*
21	3.2*	21	5.6*	22	na
28	1.2*	28	3.0*	29	na

NA = No mortality occurred in untreated control, therefore nothing could be calculated

### Secondary Conclusion (% mortality of ticks collected in crates)

Only after 24 hours of exposure to the combination product, was there cumulative % mortalities >80% on days 15 and 22 for *R. Sanguineus* and was never calculated for *D. variabilis*. This does not support any “quick” or “quickly kills” claims on the label, nor does it prove that ticks didn’t or couldn’t attach.

**MRID 47914226. Effectiveness of Two Topical Treatments one Month Apart with a Combination of ML-2,095, 988 509T and ML-3,948,906 Against Induced Infestations of *Rhipicephalus sanguineus* on Dogs.**

**Objective**

The trial objective was to determine the efficacy of the combination product when administered as its normal monthly dosing directions.

**Set Up**

Sixteen mongrel dogs > 52 weeks old were split into 2 groups of 8 dogs.

Group 1 = was a non-treated control

Group 2 = were treated with the combination product on day zero & day 28 (6.25 hours prior to infestation).

Each dog was infested with  $50 \pm 5$  ticks on days -1, 7, 14, 21, 28, 35, 42, 56, 63 & 70. Ticks were counted at 6, 18, 24 & 48 hours after day zero and at 4, 18, 24 & 48 hours after day 28. All ticks were removed 48 hours after each infestation.

**Results**

Dogs treated with the combination of chemicals had significantly ( $p \leq .05$ ) lower amounts of ticks (live and/or dead engorged) than untreated controls starting at the 18 hour mark from the initial treatment and again at every 4/6, 18, 24 and 48 hour count through day 65. Percent reductions >80% were seen on days 7-42 and at each 18, 24 & 48 hour count from day 0-65.

Efficacy Rates of % Reduction as Compared to Non-treated Control Based on Geometric means of tick counts:

(\*) indicates statistically significant.

4/6 hours		18 hours		24 hours		48 hours	
Day	% Efficacy	Day	% Efficacy	Day	% Efficacy	Day	% Efficacy
0	21	0	90.0*	1	93.9*	2	98.8*
7	96.5*	7	98.8*	8	100*	9	100*
14	95.0*	14	100*	15	100*	16	100*
21	82.5*	21	100*	22	99.2*	23	100*
28	89.0*	28	100*	29	100*	30	100*
35	89.7*	35	100*	36	100*	37	100*
42	91.8*	42	98.8*	43	100*	44	100*
49	78.3*	49	98.1*	49	98.6*	51	99.2*
56	68.5*	56	93.8*	56	94.8*	58	98.0*
63	55.6*	63	85.9*	63	88.0*	65	94.4*

**Conclusion**

The study supports label claims that the combination product will kill, control or prevent tick infestations starting at 18 hours. No “quick” or “quickly” claims are supported with this study.

## **MRID 47914227. Combination Testing of Amitraz and Fipronil in the *Rhipicephalus sanguineus* Contact Test.**

### **Objective**

To determine if amitraz has a synergist effect with fipronil.

### **Set Up**

Technical samples of both fipronil and amitraz were used and diluted to achieve desired concentrations. Vials were filled with an un-treated control, a solvent treated control, amitraz alone (in one of 3 concentrations: 6.25, 12.5 or 25 ppm), fipronil alone or a combination of amitraz and fipronil, then rolled and allowed to dry. Ten *R. sanguineus* ticks were placed in each vial and live and dead ticks were counted and recorded after 6, 24 & 48 hours.

### **Results**

There was no mortality seen in the control treatments or in the amitraz dosed at 6.25 or 12.5ppm. Only after 48 hours was there a 10% mortality reported for amitraz alone at the 25 ppm rate.

The combination of the fipronil and amitraz at the 12.5ppm rate showed synergistic ratios of x14.7, x157 & x56 at 6, 24 and 48 hours respectively, meaning there was a up to a 157-fold increase in mortality than fipronil alone. In addition, the 6 hour values of the fipronil and amitraz combos are similar to that of the fipronil alone values at 48 hours (100% mortality), indicating that the combination also provides an increased speed of kill of the ticks.

### **Conclusion**

The addition of amitraz to fipronil in the formulation improves the effectiveness of the product.

## **MRID 47914228. The Motility of Ticks Exposed to Amitraz and Fipronil Residues on a Petri Dish Using the Lemna Tec Scanalyzer Imaging System.**

### **Objective**

The trial objective was to determine the affect of amitraz, fipronil, and the combination of the two chemicals on the motility of ticks over time.

### **Set Up**

Petri dishes were treated with either an ethanol control, amitraz alone, fipronil alone (in different doses) or a combination of amitraz with different doses of fipronil. Ten *Rhipicephalus sanguineus* ticks were placed in each dish then evaluated for motility via the Scanalyzer Imaging System at 1, 4, 18-22 and 24 hours.

### **Results**

At 1 & 4 hours, both the combination of amitraz and fipronil as well as the amitraz alone treated groups had a significantly ( $p \leq .05$ ) higher motility than the fipronil alone or the control groups. At 18-22 hours the amitraz alone group had a significantly higher motility than the combo group, but at 24 hours there was no significant difference between any of the groups. At 18 hours the fipronil + amitraz group had a significantly lower motility than each other group.

## **Conclusion**

The study suggests that amitraz increases motility of ticks, which would otherwise typically be resting unless stimulated by movement or carbon dioxide.

## **RECOMMENDATIONS:**

Current claims and recommendations as they relate to the efficacy studies on ticks:

## **CLAIMS**

1. Provides synergistic efficacy against ticks
2. Long lasting
3. Rapid, Rapidly, Quick, Quickly, Fast, Fast Acting
4. Waterproof, effective after bathing, water immersion
5. Effective after exposure to sunlight
6. Prevents attachment of ticks, Prevents tick feeding
7. Causes tick paralysis
8. Rapidly kills all stages of ticks
9. Detaches ticks when applied to a dog with pre-existing infestations
10. 3-way protection against fleas, ticks and chewing lice
11. Starts killing ticks within 2 hours
12. Persistent (sustained) efficacy through 30 days
13. Proven treatment technology
14. Uses (different) (multiple) modes of action to kill (ticks)

### **Claim 1 - "*Provides synergistic efficacy against ticks*"**

Due to heightened efficacy implications, the claim is unacceptable.

**Label Recommendation – delete all**

### **Claim 2 –*Long lasting***

This claim is not qualified. In addition, claims for efficacy should coincide with label application recommendations and therefore should not be greater than 30 days for ticks, as is the instructed time for re-application.

**Label Recommendation – delete or qualify this claim to read “Lasts 30 days” to coincide with application instructions.**

### **Claim 3 –*Rapid, Rapidly, Quick, Quickly, Fast, Fast Acting***

These are not qualified claims nor is there data to support any of such claims.

Data supports the label claim “starts killing ticks in 12 hours,” which is not considered “quick” or “rapid.”

**Label Recommendation – delete all, may replace with Label “starts killing ticks in 12 hours.”**

### **Claim 4 - *Waterproof, effective after bathing, water immersion***

The data submitted supports this claim.

**Label Recommendation – Ok**

*Claim 5 - Effective after exposure to sunlight*

There were no data submitted for this claim.

**Label Recommendation – delete all such claims.**

*Claim 6 - Prevents attachment of ticks, Prevents tick feeding*

There were no data submitted for this claim.

**Label Recommendation – delete all such claims.**

*Claim 7 – Causes tick paralysis*

There were no data submitted for this claim.

**Label Recommendation – delete all such claims.**

*Claim 8 - Rapidly kills all stages of ticks*

There were no data submitted for this claim.

**Label Recommendation – delete all such claims.**

*Claim 9 - Detaches ticks when applied to a dog with pre-existing infestations*

The data submitted supports this claim.

**Label Recommendation – Ok**

*Claim 10 - 3-way protection against fleas, ticks and chewing lice*

There were no data submitted for this claim. There is no qualifying information to explain what this claim means.

**Label Recommendation – delete all such claims.**

*Claim 11 - Starts killing ticks within 2 hours*

There were no data submitted for this claim.

**Label Recommendation – delete all such claims.**

*Claim 12 - Persistent (sustained) efficacy through 30 days*

The data submitted supports this claim.

**Label Recommendation – Ok**

*Claim 13 - Proven treatment technology*  
There were no data submitted for this claim.

**Label Recommendation – delete all such claims.**

*Claim 14 - Uses (different) (multiple) modes of action to kill (ticks)*  
The data submitted supports this claim.

**Label Recommendation – Ok**