Date of Approval: July 17, 2025

FREEDOM OF INFORMATION (FOI) SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION (NADA)

NADA 141-607

Exzolt™

(fluralaner oral solution)

Laying hens and replacement chickens

Exzolt™ is indicated for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens and replacement chickens.

Sponsored by:

Intervet, Inc.

Executive Summary

Exzolt™ (fluralaner oral solution) is approved for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens and replacement chickens.

Target Animal Safety and Effectiveness

The effectiveness of Exzolt™ was demonstrated in four studies: two dose confirmation studies and two field studies. In the dose confirmation studies, Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg body weight in medicated water 7 days apart was 100% effective against northern fowl mites in laying hens and replacement chickens for up to 14 or 28 days after the first treatment. In the field studies, Exzolt™ administered using the same dosing regimen was >99.9% effective against northern fowl mites in laying hens for up to 28 days after the first treatment.

The target animal safety of Exzolt™ was evaluated in four studies: two margin of safety studies and two reproductive safety studies. In the margin of safety studies, Exzolt™ was administered to laying hens and 3-week-old broiler chickens at up to 5 times the labeled dose and 3 times the treatment duration. No clinically significant adverse effects were observed. The reproductive safety studies in layer and broiler breeder chickens administered 3 times the labeled dose showed no adverse effects on reproductive performance or offspring viability.

Human Food Safety

The human food safety evaluation of Exzolt™ was conducted through a review of a microbial food safety assessment and toxicology and residue chemistry studies. The microbial food safety assessment concluded that Exzolt™ does not pose a risk for development of antimicrobial resistance. Based on toxicology studies, the acceptable daily intake for total residue of fluralaner was established as 10 µg/kg body weight/day. The calculated safe concentrations of total residues of fluralaner were 180 parts per billion (ppb) in muscle, 540 ppb in liver, 1080 ppb in skin with fat in natural proportions, and 4260 ppb in eggs. Residue depletion studies determined that chicken liver is the target tissue and that parent fluralaner is the marker residue for monitoring residues in chicken liver, muscle, and eggs. Tolerances of 320 ppb in chicken liver, 110 ppb in chicken muscle, and 2500 ppb in chicken eggs were established. An 11-day withdrawal period for meat and a 0-day egg discard time were assigned based on residue depletion data.

User Safety

The product labeling contains precautions for people handling or administering Exzolt™. This includes wearing protective gloves, avoiding skin/eye contact and ingestion, and washing hands after use. The label states that accidental exposure may cause skin/eye irritation and accidental ingestion may cause gastrointestinal disturbances.

Conclusion

Based on the data submitted, the FDA determined that Exzolt™ is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-607

B. Sponsor

Intervet, Inc.
126 E Lincoln Ave.
Rahway, NJ 07065

Drug Labeler Code: 000061

C. Proprietary Name

Exzolt™

D. Drug Product Established Name

fluralaner oral solution

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Concentrated solution

G. Amount of Active Ingredient

10 mg fluralaner/mL

H. How Supplied

1- and 4-Liter high-density polyethylene (HDPE) plastic containers

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

Exzolt™ must be administered orally to chickens via the drinking water as 2 single doses spaced 7 days apart, with each dose consumed over a period of 6 to 24 hours. Each dose is 0.5 mg fluralaner/kg (0.227 mg/lb) body weight, equivalent to 0.05 mL of Exzolt™/kg body weight (0.023 mL/lb).

K. Route of Administration

Oral

L. Species/Classes

Chickens, laying hens and replacement chickens

M. Indication

Exzolt™ is indicated for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens and replacement chickens.

II. EFFECTIVENESS

A. Dosage Characterization

Effectiveness data generated for the approval of Exzolt™ against poultry red mites (*Dermanyssus gallinae*) in foreign countries and pilot data generated for the use of Exzolt™ against northern fowl mites (*Ornithonyssus sylviarum*), supported the decision to use two single doses of 0.5 mg fluralaner/kg body weight (bw) in medicated water 7 days apart in the studies to demonstrate substantial evidence of effectiveness for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in chickens.

B. Substantial Evidence

The sponsor conducted four studies to demonstrate substantial evidence of effectiveness of Exzolt™ for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens and replacement chickens: a dose confirmation study in replacement chickens (Study S14306-00), a dose confirmation study in laying hens (Study S19166-00), and field studies in laying hens at two different geographical locations (Studies S14379-00 and S21066-00). The studies demonstrate that two single doses of 0.5 mg fluralaner/kg bw administered in medicated water 7 days apart provide effectiveness for up to 14 days (Study S19166-00) and up to 28 days after the first treatment (Studies S14306-00, S14379-00, and S21066-00). The effectiveness duration of up to 14 to 28 days represents the typical range of time for approximately two life cycles of the northern fowl mite on the bird under different environmental conditions. In all four effectiveness studies, Exzolt™ achieved 99.3% or greater reduction in *Ornithonyssus sylviarum* mite counts on Day 2 following the first dose administration.

1. Dose Confirmation Study in Replacement Chickens

Title: Dose Confirmation Study to Confirm the Effectiveness of Fluralaner Solution (10 mg/mL) Administered Orally at Two Single Doses of 0.5 mg/kg Body Weight 7 Days Apart in the Treatment of Northern Fowl Mites (*Ornithonyssus sylviarum*) in Replacement Chickens. (Study No. S14306-00)

Study Dates: March 2015 to October 2017

Study Location: Riverside, CA

Study Design:

Objective: To confirm the effectiveness of Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart against northern fowl mites (*Ornithonyssus sylviarum*) in replacement chickens.

Study Animals: One hundred twenty-eight white leghorn (Hy-Line strain) female replacement chickens that were artificially infested with northern fowl mites were enrolled in the study. The birds were approximately 14 weeks old when the first dose was administered.

Experimental Design: Approximately 6 weeks before enrollment, each study bird was artificially infested with 50 to 100 northern fowl mites. On Day -6, the treated and control groups were randomized to 32 cages of birds in a 1:1 ratio. Each cage contained four birds with established mite infestations. Cages were formed by balancing the Day -7 mite counts. The experimental unit was the cage.

All study personnel except individuals involved in treatment assignments and treatment administration were masked. The study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Table II.1. Treatment Groups

Treatment Groups	Treatment Regimen	Number of Cages	Number of Birds per Treatment Group
Control	Non-medicated water	16	64 (4 birds/cage)
Treated	0.5 mg fluralaner/kg bw on Day 0 and Day 7 orally via medicated water	16	64 (4 birds/cage)

Drug Administration: The test article was Exzolt™, which was diluted to an appropriate concentration and administered orally via medicated water to provide a dose of 0.5 mg fluralaner/kg bw to the treated group on Day 0 and Day 7. The test article volume used for the preparation of the medicated water was calculated for each cage based on the total cage bird weight determined on Day -1 and Day 6. The volume of water was calculated based on the average daily water consumption measured for the cage on Days -4 to -3. Birds in the control group received non-medicated drinking water on Day 0 and Day 7. After all the water was consumed on Day 0 and Day 7, fresh, non-medicated drinking water was added to the waterers in all cages.

Measurements and Observations: Mite numbers were counted on all study birds by visually examining feathers and skin in an approximately 4 x 6 square cm area anterior to the vent on Days -7, 2, 8, 14, 19, and 28. Birds were examined by a veterinarian prior to the study and observed daily by study personnel for general health and presence of abnormalities.

Statistical Methods: The primary effectiveness outcome was mite vent count on Days 8, 14, 19, and 28 after the first treatment. Mite vent counts from individual birds were averaged to obtain the arithmetic mean for each cage on each count day. The arithmetic mean for each cage was transformed as $\log_e(x+1)$ and analyzed by a linear model with treatment as the fixed effect. Data were analyzed for each count day separately. Least squares (LS) means were used for treatment comparisons.

The null hypothesis was that the treatment group and control group share the same mean mite counts. The hypothesis was tested at $\alpha = 0.05$ (two-sided) significance level. The percent effectiveness at each timepoint was calculated using the formula [100 x (M_C - M_T) / M_C], where: M_C = geometric mean back-transformed from the LS mean of the control group and M_T = geometric mean back-transformed from the LS mean of the treated group.

Exzolt™ was considered effective if, on each mite counting day, the control group had an adequate infestation (defined as each bird infested with at least 25 mites and an average of ≥50 mites in the control group); percent effectiveness was ≥90%; and there was a statistically significant ($p \leq 0.05$) difference between the Exzolt™-treated group compared to the control group.

Results: On each mite count day, the control group met the adequacy of infestation criteria except for a total of five birds that had less than 25 mites on Day 19 (two birds) or Day 28 (five birds). With these deviations considered, the control group was considered to have an adequate infestation on each mite counting day. Exzolt™ was 100% effective and the mean mite counts between the two groups were significantly different ($p < 0.0001$) at 8 days after the first treatment through Day 28 (Table II.2.).

Table II.2. Comparisons of Geometric Means of Mite Counts in Treated and Control Groups

Study Day	Control (Geometric Mean*)	Treated (Geometric Mean*)	% Effectiveness	P-value†
-7	334.5	326.9	NA	NA
2	193.24	1.3	99.3	<0.0001
8	177.1	0	100	<0.0001
14	129.2	0	100	<0.0001
19	124.8	0	100	<0.0001
28	114.9	0	100	<0.0001

*For Days 2, 8, 14, 19, and 28, geometric mean was back-transformed from the LS means obtained from the statistical analysis. For Day -7, geometric mean was computed from the observed data.
†P-value was obtained from the comparison between treatment groups.

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusions: This study demonstrates that Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart is effective for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in replacement chickens.

2. Dose Confirmation Study in Laying Hens

Title: Dose Confirmation Study to Confirm the Effectiveness of Fluralaner Solution (10 mg/mL) Administered Orally at Two Single Doses of 0.5 mg/kg Body Weight 7 Days Apart in the Treatment of Northern Fowl Mites (*Ornithonyssus sylviarum*) in Laying Hens. (Study No. S19166-00)

Study Dates: March 6, 2020 to February 28, 2023

Study Location: Riverside, CA

Study Design:

Objective: To confirm the effectiveness of Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart against northern fowl mites (*Ornithonyssus sylviarum*) in laying hens.

Study Animals: One hundred twenty-eight brown leghorn (Hy-Line strain) laying hens that were artificially infested with northern fowl mites were enrolled in the study. The birds were approximately 29 weeks old when the first dose was administered.

Experimental Design: Approximately 5 weeks before enrollment, each study bird was artificially infested with 50 to 100 northern fowl mites. On Day -7, 128 healthy birds with established mite infestations were ranked by the mite counts from the highest to lowest. These 128 birds were randomly allocated to 16 units (two cages per unit and four birds per cage). On Study Day -1, the 16 units with two cages in each unit were randomly allocated to either the treated or control group, resulting in eight units (total 16 cages) per group and 64 birds per treatment group. The 2-cage unit was the experimental unit.

All study personnel except individuals involved in treatment assignments and treatment administration were masked. This study was conducted in accordance with GCP guidelines.

Table II.3. Treatment Groups

Treatment Groups	Treatment Regimen	Number of Units	Number of Cages	Number of Birds per Treatment Group
Control	Non-medicated water	8	16	64 (4 birds/cage)
Treated	0.5 mg fluralaner/kg bw on Day 0 and Day 7 orally via medicated water	8	16	64 (4 birds/cage)

Drug Administration: The test article was Exzolt™, which was diluted to an appropriate concentration and administered orally via medicated water to provide a dose of 0.5 mg fluralaner/kg bw to the treated group on Day 0 and Day 7. The test article volume used for the preparation of the medicated water was calculated for each cage based on the total cage bird weight determined on Day -1. The volume of water was calculated based on the average daily water consumption measured for the cage on Days -4 to -3. Birds in the control group received non-medicated drinking water on Day 0 and Day 7. After all the water was consumed on Day 0 and Day 7, fresh, non-medicated drinking water was added to the waterers in all cages.

Measurements and Observations: Mite numbers were counted on all study birds by visually examining feathers and skin in an approximately 4 x 6 square cm area anterior to the vent on Days -7, 2, 8, 14, 19, and 28. Birds were examined by a veterinarian prior to study and were observed daily for general health and presence of abnormalities.

Statistical Methods: The primary effectiveness outcome was mite vent count on Days 8, 14, 19, and 28 after the first treatment. Mite vent counts from individual birds were averaged to obtain the arithmetic mean for each 2-cage unit on each count day. The arithmetic mean for each 2-cage unit was transformed as $\log_e(x+1)$ and analyzed by a linear model with treatment as the fixed effect. Data were analyzed for each count day separately. Least squares (LS) means were used for treatment comparisons.

The null hypothesis was that the treatment group and control group share the same mean mite counts. The hypothesis was tested at $\alpha = 0.05$ (two-sided) significance level. The percent effectiveness at each timepoint was calculated using the formula $[100 \times (M_C - M_T) / M_C]$, where: M_C = geometric mean back-transformed from the LS mean of the control group and M_T = geometric mean back-transformed from the LS mean of the treated group.

Exzolt™ was considered effective if, on each mite counting day, the control group had an adequate infestation (defined as 6 of 8 experimental units (unit of two connected cages) having an average of ≥25 mites); percent effectiveness was ≥90%; and there was a statistically significant ($p \leq 0.05$) difference between the Exzolt™-treated group compared to the control group.

Results: The control group had an adequate infestation through Day 14. The control group did not have adequate infestations on Day 19 or 28. Exzolt™ was 100% effective at 8 and 14 days after the first treatment (Table II.4.). On all mite count days following treatment, the mean mite counts between the two groups were significantly different ($p < 0.0001$).

Table II.4. Comparisons of Geometric Means of Mite Counts in Treated and Control Groups

Study Day	Control (Geometric Mean*)	Treated (Geometric Mean*)	% Effectiveness	P-value†
-7	2235.9	1999.7	NA	NA
2	938.34	1.36	99.9	<0.0001
8	197.78	0	100	<0.0001
14	126.68	0	100	<0.0001

*For Days 2, 8, and 14, geometric mean was back-transformed from the LS means obtained from the statistical analysis. For Day -7, geometric mean was computed from the observed data.
†P-value was obtained from the comparison between treatment groups.

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusions: This study demonstrates that Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart is effective for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens.

3. Field Effectiveness Study in Laying Hens

Title: Evaluation of the Field Effectiveness and Safety of Fluralaner Solution (10 mg/mL) Administered Orally at Two Single Doses of 0.5 mg/kg Body Weight 7 Days Apart in the Treatment of Northern Fowl Mites (*Ornithonyssus sylviarum*) in Laying Hens. (Study No. S14379-00)

Study Dates: January 2016 to October 2017

Study Location: Yucaipa, CA

Study Design:

Objective: To evaluate the field effectiveness and safety of Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart against northern fowl mites (*Ornithonyssus sylviarum*) in laying hens.

Study Animals: Eight hundred white leghorn (Hy-Line strain) laying hens that were artificially infested with northern fowl mites were enrolled in the study. The birds were 23 weeks old when the first dose was administered.

Experimental Design: Approximately 4 weeks before enrollment, each study bird was artificially infested with 50 to 100 northern fowl mites. The 800 healthy birds with established mite infestations were arbitrarily assigned to 400 cages with two birds per cage. On Day 0, the two rows of cages (200 cages in each row) were randomly assigned to either the treated or control group. The experimental unit was the row of 200 cages.

All study personnel except individuals involved in treatment assignments and treatment administration were masked. This study was conducted in accordance with GCP guidelines.

Table II.5. Treatment Groups

Treatment Groups	Treatment Regimen	Number of Cages	Number of Birds per Treatment Group
Control	Non-medicated water	200	400 (2 birds/cage)
Treated	0.5 mg fluralaner/kg bw on Day 0 and Day 7 orally via medicated water	200	400 (2 birds/cage)

Drug Administration: The test article was Exzolt™, which was diluted to an appropriate concentration in a medication container and administered orally via medicated water using a dosing pump system to provide a dose of 0.5 mg fluralaner/kg bw to the treated group on Day 0 and Day 7. The test article volume used for the preparation of the medicated water was calculated for each cage row based on the total birds' weight determined on Day -1. The volume of water was calculated based on the water consumption measured for the cage row on Days -4 to -3. On Day 0 and Day 7. Birds in the control group received non-medicated drinking water on Day 0 and Day 7. After all the water was consumed on Day 0 and Day 7, fresh, non-medicated drinking water was added to the waterers in all cages.

Measurements and Observations: Mite numbers were counted on one bird per cage from 80 randomly selected cages in each group by visually examining feathers and skin in an approximately 4 x 6 square cm area anterior to the vent on Days -5, 2, 8, 14, 19, and 28. Birds were examined by a veterinarian prior to the study and were observed daily for general health and presence of abnormalities.

Statistical Methods: No statistical hypothesis tests were performed. The primary effectiveness outcome was mite vent count on Days 8, 14, 19, and 28 after the first treatment. The percent effectiveness at each timepoint was calculated using the formula [$100 \times (M_C - M_T) / M_C$], where: M_C = geometric mean of mite counts from all selected birds in the control group and M_T = geometric mean of mite counts from all selected birds in the treated group. The transformation of $\log_e(x+1)$ was used to calculate geometric means of mite counts in the control and the treated groups.

Exzolt™ was considered effective if, on each mite counting day, the control group had an adequate infestation (defined as at least 60 of the 80 birds sampled (one bird per cage) having ≥25 mites) and percent effectiveness was ≥90%.

Results: On each mite counting day, the control group had an adequate infestation. The percent effectiveness exceeded 99.9% on Days 8, 14, 19, and 28 after the first treatment (Table II.6.).

Table II.6. Comparisons of Geometric Means of Mite Counts in Treated and Control Groups

Study Day	Control (Geometric Mean)	Treated (Geometric Mean)	% Effectiveness
-5	2015.1	2178.5	NA
2	1545.2	7.7	99.5
8	1582.4	0.1	>99.9
14	1375.5	0.1	>99.9
19	3114.3	0.1	>99.9
28	2532.8	0.4	>99.9

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusions: This study demonstrates that Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart is effective for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens.

4. Field Effectiveness Study in Laying Hens

Title: Evaluation of the Field Effectiveness and Safety of Fluralaner Solution (10 mg/mL) Administered Orally at Two Single Doses of 0.5 mg/kg Body Weight, 7 Days Apart in the Treatment of Northern Fowl Mites (*Ornithonyssus sylviarum*) in Laying Hens. (Study No. S21066-00)

Study Dates: September 22, 2021 to October 4, 2023

Study Location: University Park, PA

Study Design:

Objective: To evaluate the field effectiveness and safety of Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart against northern fowl mites (*Ornithonyssus sylviarum*) in laying hens.

Study Animals: Eight hundred white leghorn (Hy-Line strain) laying hens that were artificially infested with northern fowl mites were enrolled in the study. The birds were approximately 28 weeks old when the first dose was administered.

Experimental Design: Approximately 5 weeks before enrollment, each study bird was artificially infested with 50 to 100 northern fowl mites. The 800 healthy birds with established mite infestations were arbitrarily assigned to each of the 400 cages with two birds per cage. On Day -3, the two rows of cages (200 cages in each row) were randomly assigned to either the treated or control group. The experimental unit was the row of 200 cages.

All study personnel except individuals involved in treatment assignments and treatment administration were masked. This study was conducted in accordance with GCP guidelines.

Table II.7. Treatment Groups

Treatment Groups	Treatment Regimen	Number of Cages	Number of Birds per Treatment Group
Control	Non-medicated water	200	400 (2 birds/cage)
Treated	0.5 mg fluralaner/kg bw on Day 0 and Day 7 orally via medicated water	200	400 (2 birds/cage)

Drug Administration: The test article was Exzolt™, which was diluted to an appropriate concentration in a medication tank and administered orally via medicated water using a gravity tank system to provide a dose of 0.5 mg fluralaner/kg bw to the treated group on Day 0 and Day 7. The test article volume used for the preparation of the medicated water was calculated for each cage row based on total bird weight determined on Day -1. The volume of water was calculated based on the water consumption measured for the cage row on Days -4 to -3. Birds in the control group received non-medicated drinking water on Day 0 and Day 7. After all the water was consumed on Day 0 and Day 7, fresh, non-medicated drinking water was added to the waterers in all cages.

Measurements and Observations: Mite numbers were counted on one bird per cage from 80 cages in each group by visually examining feathers and skin in an approximately 4 x 6 square cm area anterior to the vent on Days -5, 2, 8, 14, 19, and 28. Birds were examined by a veterinarian prior to study and were observed daily for general health and presence of abnormalities.

Statistical methods: No statistical hypothesis tests were performed. The primary criteria for effectiveness were mite vent counts in the treated group compared to the control group on Days 8, 14, 19, and 28 after the first treatment. The percent effectiveness at each timepoint was calculated using the formula [$100 \times (M_C - M_T) / M_C$], where: M_C = geometric mean of mite counts from all selected birds in the control group and M_T = geometric mean of mite counts from all selected birds in the treated group. The transformation of $\log_e(x+1)$ was used to calculate geometric means of individual bird mite counts in the control and the treated groups.

Exzolt™ was considered effective if, on each mite counting day, the control group had an adequate infestation (defined as at least 60 of the 80 birds sampled (one bird per cage) having ≥25 mites)) and percent effectiveness was ≥90%.

Results: On each mite counting day, the control group had an adequate infestation. The percent effectiveness exceeded 99.9% on Days 8, 14, 19, and 28 after the first treatment (Table II.8.).

Table II.8. Comparisons of Geometric Means of Mite Counts in Treated and Control Groups

Study Day	Control (Geometric Mean)	Treated (Geometric Mean)	% Effectiveness
-5	422.0	855.5	NA
2	477.7	1.9	99.6
8	865.6	0.2	>99.9
14	289.9	0	100
19	358.7	0.1	>99.9
28	213.8	0.1	>99.9

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusions: This study demonstrates that Exzolt™ administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart is effective for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens.

III. TARGET ANIMAL SAFETY

The target animal safety of the labeled dosage of Exzolt™ for laying hens and replacement chickens was evaluated in four studies: two margin of safety studies and two reproductive safety studies. The margin of safety was evaluated in laying hens and 3-week-old broiler chickens. In these studies, Exzolt™ was administered orally via drinking water at 0.5, 1.5, or 2.5 mg fluralaner/kg bw/day (1X, 3X, or 5X the labeled dose) on six separate days (Days 1, 8, 15, 22, 29, and 36 in the broiler chicken margin of safety study; and Days 1, 2, 3, 8, 9, and 10 in the laying hen margin of safety study). In the reproductive safety studies, Exzolt™ was administered orally via drinking water to layer breeder chickens and broiler breeder chickens at 1.5 mg fluralaner/kg bw/day (3X the labeled dose) on four occasions, seven days apart, covering at least one ovulation cycle in females (1 day), egg formation (12 days) and a full spermatogenic cycle in the males (13 days).

A. Margin of Safety Study in Laying Hens

Title: Pivotal Margin of Safety Study of Fluralaner in Chickens. (Study No. S14123-00)

Study Dates: October 2014 to June 2016

Study Location: Tranent, Edinburgh, United Kingdom

Study Design:

Objective: To evaluate the safety of Exzolt™ to laying hens when administered orally via drinking water at 1X, 3X, and 5X the recommended label dose (0.5, 1.5, and 2.5 mg fluralaner/kg body weight) for three consecutive days (3X duration), seven days apart (Days 1, 2, and 3 and Days 8, 9, and 10). The label dosage is two single doses of 0.5 mg fluralaner/kg bw administered in medicated water 7 days apart (total dose of 1 mg fluralaner/kg bw).

Study Animals: One hundred twenty healthy commercial Novogen laying hens, laying on average five good quality eggs per week were enrolled in the study. The birds were approximately 28 weeks old when the dosing period started. The body weight of the birds at enrollment ranged from 1.403 to 1.997 kg.

Experimental Design: This was a randomized, masked, nonclinical laboratory study, conducted in compliance with the Good Laboratory Practice (GLP) regulations (21 CFR Part 58). One hundred twenty individually housed hens were randomly assigned to one of the four treatment groups (control group and 1X, 3X, and 5X treatment groups) as follows. Thirty blocks of pens were formed based on the facility diagram such that four adjacent pens formed a block. Birds were grouped based on similar bw (four birds in each group) and randomly assigned to blocks and pens within blocks. Within each block, birds were randomly assigned to one of the four treatment groups. Each treatment group contained 30 hens.

After being randomized to treatment groups, birds within a treatment group were randomly allocated to one of four categories:

- Four birds were assigned as blood sampling birds.
- Eight birds were assigned as necropsy birds.
- Eight birds were assigned as blood sampling and necropsy birds.
- Ten birds were assigned for egg evaluation.

Drug Administration: The test article was Exzolt™, which was administered to the 1X, 3X, and 5X groups in medicated water over 24-hour periods on Days 1, 2, 3, 8, 9, and 10 at target doses of 0.5, 1.5, and 2.5 mg/kg bw/day, respectively. The test article volume used for the preparation of the medicated water for each treatment group was calculated based on the total body weight of all birds in the treatment group measured on Day -1 (for treatment on Days 1, 2, and 3) and Day 7 (for treatment on Days 8, 9, and 10), and the mean daily water consumption for the treatment group over five days before treatment on Day 1 (Day -6 to Day -2) and Day 8 (Day 2 to Day 6), respectively. The control (0X) group received non-medicated drinking water during the dosing period. The mean daily dose levels achieved for each treatment group were within five percent of the target daily dose level of 0, 0.5, 1.5, and 2.5 mg fluralaner/kg bw.

Table III.1. Treatment Groups

Treatment Groups	Number of Pens	Number of Birds/Pen	Treatment	Treatment Days
Control	30	1	Non-medicated drinking water	Days 1, 2, 3 and Days 8, 9, 10
1X	30	1	0.5 mg fluralaner/kg bw	Days 1, 2, 3 and Days 8, 9, 10
3X	30	1	1.5 mg fluralaner/kg bw	Days 1, 2, 3 and Days 8, 9, 10
5X	30	1	2.5 mg fluralaner/kg bw	Days 1, 2, 3 and Days 8, 9, 10

Measurements and Observations: Individual birds were observed twice daily to monitor birds' health and once daily to monitor the environment (temperature and humidity). Twice daily health assessments included litter moisture and excreta, body condition, eyes, respiration, nasal discharge, locomotion/muscular, skin and feathers, and behavioral attitude.

Clinical observations were performed by a veterinarian on Days -2, 4, 11, 29, and 36. Each bird was examined for abnormalities in general appearance and behavior, and in the musculoskeletal system, respiratory system, and integumentary system. A withdrawal reflex test was also performed on each bird to test sensory and motor function.

Daily feed consumption and water intake were recorded for all individual birds from Day -7 until euthanasia. The individual bird's body weight was taken prior to randomization and on Days -8, -1, 4, 7, 11, 18, 25, 29, and 36.

Eggs were collected twice daily from each pen. Egg production and egg abnormalities, such as soft shells or misshapen eggs, were evaluated for all hens. Eggs collected from the 10 pre-selected hens per treatment group were evaluated for the egg quality parameters: soundness of shell, egg height: width ratio (egg shape), eggshell thickness, egg strength, egg weight, albumin height, yolk color, Haugh unit, and presence of blood and meat spots.

The birds selected for hematology or clinical chemistry evaluations were subject to blood sampling on Days -3, 4, 11, 21, and 29. Reference intervals were generated using previously collected data from separate healthy laying hens of a similar strain (Hy-line) and age that were managed the same as the study birds (housing, nutrition, light/dark cycle, environmental temperatures). Blood samples used to generate reference intervals were analyzed in the same laboratory and with the same equipment and analytical methodology as the blood samples in Study S14123-00. Gross necropsies and tissue collection for histopathological examination were performed on eight pre-selected birds per treatment group on Day 11 and eight pre-selected birds per treatment group on Day 29. The remaining 14 birds per treatment group remained on study for an additional recovery period of seven days and were euthanized at the end of the study (Day 36).

Histopathologic examinations of all collected tissues were only performed on the birds from the control and 5X treatment groups. Histopathologic examinations were not performed on the birds from the 1X and 3X treatment groups because no abnormal findings were noted in the 5X treatment group that warranted further examination in the 1X and 3X treatment groups.

Statistical Methods: The experimental unit was the individual bird. Body weights, feed consumption, water intake, egg production, egg strength, egg weight, egg shell thickness, Haugh unit, and clinical pathology data collected during the treatment phase of the study (from acclimatization to Day 11) were analyzed using repeated measures analysis of covariance (RMANCOVA) with time, treatment, and treatment-by-time interaction as fixed effects; pre-treatment value as the covariate; and animal ID as a random effect. The RMANCOVA model with best fitted covariance structure was applied. For data collected during the recovery phase 1 (Day 11 to Day 29), the repeated measures analysis of variance (RMANOVA) was applied using the same model as RMANCOVA but without the covariate. For data collected during the recovery phase 2 (Day 29 to Day 36), the analysis of variance (ANOVA) was applied using treatment as the fixed effect. All fixed effects were tested at $\alpha = 0.10$ (two-sided when applicable). No adjustment was made for multiple comparisons. Summary statistics were used to generate clinical pathology reference intervals.

Results: No abnormalities or mortalities were recorded for any bird during daily health observations and clinical observations after the treatment administration.

There were no treatment-related effects on water intake, egg production, and egg quality parameters. The Exzolt™-treated groups (1X and 3X) had statistically significantly different and increased daily feed consumption compared to the control group during the recovery phase (Days 11 to 29) (Table III.2.) and the Exzolt™-treated group (3X) had statistically significantly different and increased body weight on Days 7 and 11 when compared with the control group (Table III.3.). However, these differences were not considered an adverse effect of treatment because feed consumption and body weight were increased for these groups and timepoints as compared to the control group and no dose related trends were observed.

Table III.2. LS Mean Daily Feed Consumption (Kg) in the Control and Treated Groups During the Treatment and Recovery Phases

Treatment Groups	Treatment Phase (Days 1 to 11)	Recovery Phase 1 (Days 11-29)	Recovery Phase 2 (Days 29-36)
Control	0.111	0.104	0.103
1X	0.112	0.111*	0.108
3X	0.114	0.108†	0.107
5X	0.111	0.107	0.107

* P=0.008
† P=0.094

Table III.3. LS Mean Body Weight (Kg) in the Control and Treated Groups During the Treatment and Recovery Phases

Treatment Groups	Treatment Phase (Day 4)	Treatment Phase (Day 7)	Treatment Phase (Day 11)	Recovery Phase 1 (Days 18, 25, 29)	Recovery Phase 2 (Day 36)
Control	1.776	1.763	1.774	1.766	1.753
1X	1.774	1.773	1.772	1.833	1.790
3X	1.782	1.783*	1.795†	1.788	1.786
5X	1.767	1.774	1.766	1.767	1.756

* P=0.015
† P=0.063

There was no statistically significant difference between control and treated groups for hematology and clinical chemistry parameters except for monocytes, calcium, albumin, and glutamate dehydrogenase. There were no statistically significant differences or changes in clinical pathology variables that were considered potentially treatment-related because the differences were not biologically meaningful and did not show consistent dose dependent trends in treated animals as compared to the control group. In addition, any deviations from the reference interval in individual birds were of low magnitude, sporadic, and did not worsen with continued drug exposure.

No treatment related effects were observed during gross necropsy or histopathology evaluations for either the treatment or recovery phases. Because no treatment related histopathological findings were recorded in the 5X group histopathology evaluations were not performed in the 1X and 3X groups.

Conclusion: The study demonstrates that Exzolt™ is safe for use in laying hens when administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart.

B. Margin of Safety Study in 3-Week-Old Broiler Chickens

Title: Pivotal Margin of Safety Study of Fluralaner in 3-Week-Old Chickens. (Study No. S15327-00)

Study Dates: November 2015 to July 2016

Study Location: Tranent, United Kingdom

Study Design:

Objective: To evaluate the safety of Exzolt™ when administered orally via drinking water to 3-week-old chickens at 1X, 3X, and 5X the recommended label dose for a 3X duration on six separate days, seven days apart (Days 1, 8, 15, 22, 29, and 36). The label dosage is two single doses of 0.5 mg fluralaner/kg bw administered in medicated water 7 days apart (total dose of 1 mg fluralaner/kg bw).

Study Animals: Three hundred twenty healthy Ross 308 parent stock strain chicks (160 male and 160 female) were enrolled in the study. The birds were 21 or 22 days old, and the average individual bird body weight per pen ranged from 305 to 539 g on Day -1.

Experimental Design: This was a randomized, masked, nonclinical laboratory study, conducted in compliance with the GLP regulations (21 CFR Part 58).

Three hundred twenty birds (160 male and 160 female) were randomly allocated into pens of 8 birds each, with 10 pens (5 male and 5 female pens) in each of the four treatment groups (control group and 1X, 3X, and 5X treatment groups). One bird in the 1X treatment group and two birds in the 3X treatment group initially assigned as males were later found to be females, and this information was considered during the analysis of data.

Within each sex, pens were randomly assigned to one of four treatment groups such that five pens were assigned to each treatment group. Birds within each pen were randomly allocated to one of six categories as follows:

- One bird per pen was assigned to hematology sampling on Study Days -6, 2, 23, 37, 47, and 55.
- One bird per pen was assigned to hematology sampling on Study Day -4 to provide data to generate pre-dose reference data.
- One bird per pen was assigned to clinical chemistry sampling on Study Days -6, 2, 23, 37, 47, and 55.
- One bird per pen was assigned to clinical chemistry sampling on Study Day -4 to provide data to generate pre-dose reference data.
- One bird per pen was assigned to gross necropsy on Study Day 37.
- One bird per pen was assigned to gross necropsy on Study Day 55.

The remaining two birds per pen were available to be used as spare blood sampling birds if a bird assigned to hematology or clinical chemistry sampling was unable to be bled pre-dose only. The bird blood sampled pre-dose was also blood sampled for the remainder of the study; another bird could not replace a bird assigned to blood sampling after Study Day -6.

Drug Administration: The test article was Exzolt™, which was administered in medicated drinking water over a 24-hour period on Days 1, 8, 15, 22, 29, and 36 at target doses of 0.5, 1.5, and 2.5 mg/kg bw. The test article volume used for the preparation of the medicated water for each treatment group was calculated based on the total body weight of all birds in the treatment group measured on the day before treatment, and the mean daily water consumption for the treatment group over the five days before each treatment day. The control (0X) group received non-medicated drinking water during the dosing period. The mean daily dose levels achieved for control, 1X, 3X, and 5X treatment groups were 0, 0.55, 1.75, and 3.07 mg fluralaner/kg bw, respectively.

Table III.4. Treatment Groups

Treatment Groups	Number of Pens	Number of Birds/Pens	Treatment	Treatment Days
Control	10	8	Non-medicated drinking water	Days 1, 8, 15, 22, 29, and 36
1X	10	8	0.5 mg fluralaner/kg bw	Days 1, 8, 15, 22, 29, and 36
3X	10	8	1.5 mg fluralaner/kg bw	Days 1, 8, 15, 22, 29, and 36
5X	10	8	2.5 mg fluralaner/kg bw	Days 1, 8, 15, 22, 29, and 36

Measurements and Observations: Individual birds and pens were observed twice daily to monitor birds' health and once daily to monitor the environment (temperature and humidity). Twice daily health assessments included litter moisture and excreta, body condition, eyes, respiration, nasal discharge, locomotion/muscular, skin and feathers, and behavioral attitude. Birds showing abnormal signs were examined by a veterinarian.

Clinical observations were performed by a veterinarian on Days -10, 2, 37, and 55. Each bird was examined for abnormalities in general appearance and behavior; and in the musculoskeletal system, respiratory system, and integumentary system. A withdrawal reflex test was also performed on each bird to test sensory and motor function.

Daily feed consumption and water intake were recorded for all pens from Day -7/-8 until euthanasia. The individual bird's body weight was recorded on Days -20/-19, -14/-13, 37, 42/43, 49/50, and 55. Pen body weight was recorded on Days -1, 7, 14, 21, 28, and 35 for dose calculation.

The birds selected for hematology or clinical chemistry evaluations were subject to blood sampling on Days -6, -4, 2, 23, 37, 47, and 55. Reference intervals were generated concurrent with the study using baseline data from 88 birds that were selected for hematology or clinical chemistry evaluations or that were spare birds for the study (44 for hematology and 44 for clinical chemistry).

Gross necropsies and tissue collection for histopathological examination were performed on 10 pre-selected birds from each treatment group on Day 37 and Day 55. Histopathologic examinations were performed on all pre-selected birds from the control and 5X treatment groups, in addition to 13 birds in the 1X treatment group, and 3 birds in the 3X treatment group for which tissues were examined at the discretion of the pathologist. Because no treatment related histopathological findings were recorded in the 5X group, full histopathology evaluations were not performed in all preselected 1X and 3X group birds.

Statistical Methods: The experimental unit was the pen. Body weights, feed consumption, water intake, and clinical pathology collected during the treatment phase (from acclimation to Day 36) of the study were analyzed using RMANCOVA with time, treatment, sex, and their two-way and three-way interactions as fixed effects; pre-treatment values as the covariate; and pen as a random effect. The RMANCOVA model with best fitted covariance structure was applied. For data collected during the recovery phase (Days 37 to 55), RMANOVA was applied using the model with the same fixed and random effects as in RMANCOVA but without the covariate. The three-way interaction was tested at $\alpha = 0.05$ and all other fixed effects were tested at $\alpha = 0.10$ (two-sided, when applicable). No adjustment was made for multiple comparisons. Summary statistics were used to generate clinical pathology reference intervals.

Results: Abnormalities were observed for several birds during the daily health observations and clinical observations after the treatment administration. The abnormalities noted were stiff gait, lameness, lethargy, difficulty in respiration, and feather loss, and bleeding due to fighting. The number of abnormal birds and the abnormalities noted in the control and treated groups were similar.

Four birds (three birds in the control group and one bird in the 3X treatment group) died during the study. The dead birds from the control group had labored respiration, hepatocellular necrosis with liver inflammation, and enlarged spleen. The dead bird from the 3X group had bone marrow necrosis with inflammation. Bone marrow related lesions were not found in any other birds from 1X, 3X, and 5X groups. There was no mortality in the 1X and 5X treatment groups.

There were no treatment related effects on water intake, food consumption, or body weight throughout the study.

During the treatment phase of the study (Days 2, 23, and 37), statistically significant differences between the control and treated groups were noted for the following clinical pathology parameters: mean corpuscular volume, mean corpuscular hemoglobin, monocytes, aspartate aminotransferase, chloride, total protein, albumin, and uric acid. In addition, during the recovery phase of the study (Day 47 and Day 55), statistically significant differences between the control and treated groups were noted for the following clinical pathology parameters: white blood cell counts, heterophils, lymphocytes, eosinophils, sodium, chloride, total protein, albumin, globulin, calcium, phosphate, and uric acid. Among the parameters for which statistically significant differences between the control and treated groups were reported, variations from the normal reference interval were noted in individual animals for lymphocytes, total protein, and globulin. The changes were not considered potentially treatment related because the differences between treatment groups were not biologically meaningful and did not show dose dependent trends in treated animals as compared to the control group. In addition, deviations from the reference interval in individual birds were of low magnitude, sporadic, and did not worsen with continued drug exposure.

No treatment related effects were observed during gross necropsy or histopathology evaluations for either the treatment or recovery phases.

Conclusion: The study demonstrates that Exzolt™ is safe for use in 3-week-old broiler strain chickens when administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart.

C. Reproductive Safety Study in Layer Breeder Chickens

Title: Fluralaner Solution (1% w/v): Drinking Water Administered Reproduction Safety Study in Layer Chicken Breeders. (Study No. S14325-00)

Study Dates: November 2015 to October 2016

Study Location: Tulare, CA

Study Design:

Objective: To evaluate the reproductive safety of Exzolt™ for layer breeder chickens at approximate peak egg production when administered at a dose of 1.5 mg fluralaner/kg (3X the labeled daily dose) orally via drinking water on four separate days, seven days apart. This treatment period covered at least one estrous cycle in females (1 day), egg formation (12 days), and a full spermatogenic cycle in the males (13 days).

Study Animals: Four hundred thirty-two birds (48 male and 384 female) layer breeders (Bovans Brown) were enrolled in the study based on pre-treatment reproductive performance (percent hatchability and subsequent egg production). The body weight of the males and females, approximately 17 weeks old at study inclusion, ranged from 1.90 to 2.05 kg (Day 0) and 1.35 to 1.45 kg (Day 4), respectively. The birds were approximately 31 weeks old when the dosing period started.

Experimental Design: This was a randomized, masked, nonclinical laboratory study, conducted in compliance with the GLP regulations (21 CFR Part 58).

Three males and 24 females were randomly assigned, on Day 0 and Day 4 respectively, to each of 24 pens (total of 72 males and 576 females) and acclimated for the study. The 24 pens of birds were raised according to the layer breeder management manual for the strain selected. On Day 91, 16 pens were selected based on pre-treatment reproductive performance and randomly assigned to the control and treated groups (eight control and eight treated pens). Prior to dosing, one male and two females per pen were randomly selected for necropsy on Day 126. On the hatch day following the Day 91 to 98 and Day 119 to 126 egg collection periods (Days 120 and 148, respectively), two males and two females of newly hatched chicks per pen were randomly selected. These 64 chicks were brought to a new single pen for 14-day viability evaluations.

Drug Administration: The test article was Exzolt™ which was administered to the 3X group for an approximately 6-hour period on Days 98, 105, 112, and 119 as medicated drinking water to deliver a target dose of 1.5 mg fluralaner/kg bw. The test article volume used for the preparation of the medicated water was calculated using pen water consumption for the two days prior to dosing and the body weights on Days 98, 105, 112, and 119. The control (0X) group received regular drinking water during the dosing period. The mean daily dose levels achieved for the 3X group was

1.4 mg fluralaner/kg bw (2.8X).

Table III.5. Treatment Groups

Treatment Groups	Number of Pens	Number of Birds/Pens	Treatment	Treatment Days
Control	8	3 males and 24 females	Non-medicated drinking water	Days 98, 105, 112, and 119
3X	8	3 males and 24 females	1.5 mg fluralaner/kg bw	Days 98, 105, 112, and 119

Measurements and Observations: Clinical observations of the birds were performed twice daily starting on Day 0 to monitor birds' general health. The clinical observations included documentation of mortality, and an external examination of the feathers, eyes, and beaks, as well an examination for any abnormalities in behavior and locomotion. Similar clinical observations were performed twice daily on the newly hatched chicks that were selected for a viability evaluation at 14 days of age. The chicks were only observed once on the day of hatch.

Pen feed consumption was recorded daily from Day 91 through Day 125. Pen water consumption was recorded daily starting on Day 91 and throughout the remainder of the study. The birds were weighed by pen on Days 91, 98, 105, 112, 119, and 126. In addition, as part of the reproductive evaluations, newly hatched chicks and 14-day-old chicks were individually weighed.

Eggs were collected daily from each pen, weighed, and any egg abnormalities recorded throughout the study. Fertility (at incubation Day 7), hatchability (on incubation Day 21), and 14-day-old chick viability were evaluated from the eggs collected from Days 119 to 125. A 21-day incubation period followed each egg collection period. A gross evaluation was performed of unhatched eggs. Data collected prior to the initiation of dosing were used as covariates for the statistical analyses, where appropriate.

The fertility and hatchability outcomes were defined as follows:

> Fertility of total (%) = (number of fertile eggs* / number of eggs transferred for incubation) x 100
>
> *Fertile eggs = number of early death eggs + number of viable eggs transferred to hatcher
>
> Hatchability of total (%) = (number of normal chicks / number of eggs transferred for incubation) x 100
>
> Hatchability of viable (%) = (number of normal chicks / number of viable eggs transferred to hatcher) x 100

Gross necropsies were performed on one male and two females per pen on pre-selected birds on Day 126 and macroscopic and microscopic evaluations of reproductive organs were performed.

Statistical Methods: The pen was the experimental unit for most evaluation except for 14-day-old chick weight and viability, where the individual chick was the experimental unit. For continuous variables that were repeatedly measured (e.g., body weights, egg production and egg weights) the data were analyzed using RMANCOVA with treatment, week and treatment-by-week interaction as fixed effects, pen as a random effect, and the pre-dosing value as the covariate. The best fitted covariance structure was applied. For binomial variables (e.g., fertility, hatchability, and most of the chick viability parameters), the data were analyzed using the logit linear mixed model with treatment as a fixed effect, pen as a random effect, and the pre-dosing value as the covariate. The 14-day-old chick weights were analyzed using the ANOVA with treatment, sex, and the treatment-by-sex interaction as fixed effects. There were no mortalities in the 64 chicks selected for the 14-day viability assessment, so no analysis was performed. All fixed effects were tested at $\alpha = 0.10$ (two-sided when applicable). No adjustment was made for multiple comparisons.

Results: No abnormalities or mortalities were recorded for any bird during daily clinical observations after the treatment administration.

There were no treatment-related effects on adult bird body weight, water consumption, feed consumption, egg production, egg weights, fertility, hatchability, chick weight, and 14-day-old chick viability. The 3X group had statistically significantly different ($p = 0.0932$) and higher female reproductive tract to brain weight ratios (39.2:1) than the control group (35:1). The higher female reproductive tract to brain weight ratio in the 3X group was not associated with any corresponding macroscopic or microscopic pathology findings and there was no statistically significant difference between groups in absolute female reproductive tract weights. The 3X group (LS mean weight 25.31 g) had statistically significantly different testes weights than the control group (LS mean weight 30.76 g). There were no macroscopic or microscopic pathological findings in the testes in either the 3X group or the control group and no statistically significant difference in the testis weight to brain weight ratio between the treatment groups. Testes weights in the control group ranged from 21.3 to 40.1 grams and in the 3X group ranged from 18.1 to 32.8 grams. These weights are within the testes weight range reported in published literature for roosters with comparable age, body weight, and breed and the difference between groups is not considered clinically relevant.

Conclusion: The study demonstrates that Exzolt™ is safe for use in layer breeder chickens when administered as two single doses of 0.5 mg fluralaner/kg bw in medicated water 7 days apart and supports safety for layer breeder and laying hen replacement chickens.

D. Reproductive Safety Study in Broiler Breeder Chickens

Title: Fluralaner 10 mg/mL Oral Solution: Target Animal Reproduction Safety Study in Broiler Breeders Following Oral Administration via Drinking Water. (Study No. S19089-00)

Study Dates: January 2020 to December 2021

Study Location: Tulare, CA

Study Design:

Objective: To evaluate the reproductive safety of Exzolt™ for broiler breeder chickens at approximate peak egg production when administered at a dose of 1.5 mg fluralaner/kg bw/day (3X the labeled dose) orally via drinking water on four occasions, seven days apart. The treatment period covered at least one estrous cycle in females (1 day), egg formation (12 days), and a full spermatogenic cycle in the males (13 days).

Study Animals: Four hundred thirty-two birds (48 male and 384 female) broiler breeder chickens (Cobb 500 Fast Feathering Strain) were enrolled in the study at 21 to 22 weeks of age based on pre-treatment reproductive performance (percent hatchability and subsequent egg production). The average body weight of the males (Day 0) and females (Day 7) at enrollment was 2.7 and 2.8 kg, respectively. The birds were approximately 32 weeks (males) and 33 weeks (females) old when the dosing period started.

Experimental Design: This was a randomized, masked, nonclinical laboratory study, conducted in compliance with the GLP regulations (21 CFR Part 58).

Three males and 24 females were randomly assigned on Day 0 and Day 7, respectively, to each of the 24 pens (total of 72 males and 576 females) and acclimated to the study. The 24 pens of birds were raised according to the layer breeder management manual for the strain selected. On Day 72, 16 pens were selected based on pre-treatment reproductive performance and randomly assigned to the control and treated groups (eight control and eight treated pens). Prior to dosing, one male and two females per pen were randomly selected for necropsy on Day 105. On the hatch day following the Day 70 to 76 and Day 98 to 104 egg collection periods (Days 98 and 126, respectively), 32 chicks per treatment group (two males and two females per pen) were randomly selected. These 64 chicks were brought to a new single pen for 14-day viability evaluations.

Drug Administration: The test article was Exzolt™ which was administered to the 3X group over a period of approximately 24 hours ± 30 minutes on Days 77, 84, 91, and 98 as medicated drinking water at a target dose of 1.5 mg fluralaner/kg bw/day. The test article volume used for the preparation of the medicated water was calculated using pen water consumption for the two days prior to dosing and the body weights on the day before dosing (Days 76, 83, 90, and 97). The control (0X) group received regular drinking water during the dosing period. The mean daily dose levels achieved for the treated group was 1.54 mg fluralaner/kg bw (3.08X).

Table III.6. Treatment Groups

Treatment Groups	Number of Pens	Number of Birds/Pens	Treatment	Treatment Days
Control	8	3 male and 24 female	Non-medicated drinking water	Days 77, 84, 91, and 98
3X	8	3 male and 24 female	1.5 mg fluralaner/kg bw	Days 77, 84, 91, and 98

Measurements and Observations: General health observations of the birds were performed twice daily to monitor birds' general health. Daily health assessments included external examination of the feathers, eyes, and beaks, as well as any abnormalities in behavior, locomotion, and documentation of mortality. Similar general health observations were made on the newly hatched chicks that were selected for a viability evaluation at 14 days of age. The chickens were only observed once on the day of hatch.

Pen feed consumption was recorded every other day starting on Day 0 and throughout the remainder of the study. Pen water consumption was recorded daily starting on Day 70 and throughout the remainder of the study.

During the acclimation period (Study Days 0 through 76) and for the remainder of the study (Days 83, 90, 97, and 105), animals were weighed weekly by sex by pen for feed allocation determination. In addition, as part of the reproductive evaluations, newly hatched chicks and 14-day-old chicks were individually weighed.

Eggs were collected daily from each pen, weighed and any egg abnormalities recorded throughout the study. Fertility (at incubation Day 7) and hatchability (on incubation Day 21), and 14-day-old chick viability were evaluated from the eggs collected from Days 98 to 104. A 21-day incubation period followed each egg collection period. A gross evaluation was performed of unhatched eggs. Data collected prior to the initiation of dosing were used as covariates for the statistical analyses, where appropriate.

The fertility and hatchability outcomes were defined as follows:

> Fertility of total (%) = (number of fertile eggs* / number of eggs transferred for incubation) x 100
>
> *Fertile eggs = number of early death eggs + number of viable eggs transferred to hatcher
>
> Hatchability of total (%) = (number of normal chicks / number of eggs transferred for incubation) x 100
>
> Hatchability of viable (%) = (number of normal chicks / number of viable eggs transferred to hatcher) x 100

Gross necropsies were performed on one male and two females per pen on pre-selected birds on Day 105 and macroscopic and microscopic evaluations of reproductive organs were performed.

Statistical Methods: The pen was the experimental unit for most evaluations, except for binomial variables of produced eggs and 14-day-old chick weight and viability, where the individual egg and chick were the respective experimental units. For continuous variables that were repeatedly measured (e.g., body weights, egg production, and egg weights) the data were analyzed using RMANCOVA with treatment, week, and treatment-by-week interaction as fixed effects, pen as a random effect, and the pre-dosing value as the covariate. For binomial variables (e.g., fertility, hatchability, and most of the chick viability parameters) the data were analyzed using the logit linear model with treatment as a fixed effect and the pre-dosing value as the covariate. The 14-day-old chick weights were analyzed using the ANOVA with treatment, sex, and the treatment-by-sex interaction as a fixed effect, and pen as a random effect. All fixed effects were tested at $\alpha = 0.10$ (two-sided when applicable). No adjustment was made for multiple comparisons.

Results: No abnormalities or mortalities were recorded for any bird during daily health observations after the treatment administration.

There were no treatment-related effects on adult bird body weight, feed consumption, egg production, egg weights, fertility, hatchability, and 14-day-old chick weights, and 14-day-old chick viability. The 3X group had statistically significantly different and lower water consumption when compared with the control group on Study Days 80, 84, 91, 92, and 98,

The differences between the treatment groups are not considered clinically relevant because they were negligible compared to the day to day variation in water consumption within each treatment group. The water consumption fluctuated in both groups during the study, likely due to the skip-a-day feeding regimen used in the study and the temperature (30 to 90°F) and humidity (26 to 95%) changes in the barn. These fluctuations are expected for commercial broiler breeding chickens under these conditions. Even with the reduction in water consumption in the treated group, the treated group received the appropriate dose (1.54 mg fluralaner/kg bw; 3.08X).

There were no treatment-related effects on male and female reproductive tract weights, and no treatment-related effects were found during necropsy and histopathology evaluations.

The 3X group (47.73 g) had statistically significantly different Day 0 chick weights when compared with the control group (47.14 g). However, Day 14 chick weights were not statistically significantly different between the control and 3X group. All chicks remained healthy throughout the 14-day evaluation period. Chick weights (Day 0 and Day 14) were within the expected weight ranges for this strain of bird.

Conclusion: The study demonstrates that Exzolt™ is safe for use in broiler breeder chickens when administered as two single doses at 0.5 mg fluralaner/kg bw via drinking water seven days apart and supports safety for broiler breeder replacement chickens.

IV. HUMAN FOOD SAFETY

A. Microbial Food Safety

The Agency evaluated microbial food safety for the use of Exzolt™ (fluralaner oral solution) for the treatment and control of northern fowl mites (*Ornithonyssus sylviarum*) in laying hens and replacement chickens. The submitted microbial food safety assessment included a hazard characterization describing the use of Exzolt™ in the poultry environment and the potential to select for the emergence or dissemination of antimicrobial resistance among organisms of public health concern originating from chickens treated with Exzolt™. The sponsor also provided the information demonstrating that fluralaner has poor antibacterial activities against selected bacteria of public health concern, including *Enterococcus* species, *Campylobacter* species, *Escherichia coli*, and *Salmonella*.

Based on review of information submitted and available in the public domain, the Agency determined:

- Fluralaner is not indicated for the treatment of a bacterial disease in food producing animals;
- Fluralaner is not regularly considered to have properties that would exert antimicrobial resistance pressure towards the emergence or selection of bacteria of public health concern;
- Fluralaner is not used to treat zoonotic gastroenteritis or other bacterial disease in humans; and
- Fluralaner (or a similar compound) is not under development to treat bacterial diseases in humans.

Therefore, the Agency concluded that the proposed use of Exzolt™ in chickens does not pose a microbial food safety risk to human health with respect to antimicrobial resistance.

B. Toxicology

1. Summary of Toxicology Studies

Toxicity tests that characterized the toxicity of fluralaner (also referred to as carbamoyl benzamide phenyl isooxazoline or CBPI) in laboratory animals are summarized below.

a. Subchronic Oral Toxicity Studies in Rodents

(1) Study 1

Title: 13-Week Oral (Gavage) Toxicity Study in the Wistar Rat with Carbamoyl Benzamide Phenyl Isoxazoline (CBPI). (Study No. C45184)

Report Date: January 4, 2013

Study Location: Itingen, Switzerland

Study Design: In this oral toxicity study (GLP-compliant and following Organization for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals No. 408 (Test Guideline 408)), CBPI (purity 99.6%) was administered daily via gavage to Wistar rats at dose levels of 20, 40, or 400 mg/kg bw/day for a period of 13 weeks (10 rats/sex/group). A control group was treated with vehicle only (0.5% carboxymethylcellulose (w/v) aqueous solution containing 0.1% polysorbate 80 (v/v)). A satellite group were used for toxicokinetic evaluations, 9 rats/sex/group (3 in the control group). General cage-side observations were recorded at least once daily. Clinical observations, food consumption, body weights, estrus stage, functional observational battery (FOB) including measurement of grip strength and locomotor activity, ophthalmological examinations, hematology, clinical chemistry, and urinalysis were conducted. Animals were necropsied and examined macroscopically. Select organ weights were recorded and histological examinations were performed on organs, tissues, and lesions.

Results and Conclusion: No CBPI-related mortalities were observed during the study. One female from the toxicokinetic group was found dead on treatment day 31. No clinical signs or effects on body weight were observed in this animal before its premature death. The cause of death could not be established.

There were test article-related effects on hematology parameters. At 400 mg/kg bw/day dose level, the following changes were considered treatment-related: 1) increased hemoglobin concentration distribution width in males and females, 2) decreased relative lymphocyte counts in males, 3) increased relative neutrophil counts in males, and 4) increased absolute neutrophil counts in males. At 40 mg/kg bw/day dose level, the following changes were considered treatment-related: 1) increased hemoglobin concentration distribution width in males, 2) decreased relative lymphocyte counts in males, and 3) increased relative neutrophil counts in males.

There were test article-related effects on clinical chemistry parameters. In the 400 mg/kg bw/day dose group the following changes were considered treatment-related: 1) decreased phospholipid concentration in males and decreased phospholipid concentration in females, 2) moderately decreased triglyceride concentration in females, 3) decreased total protein concentration in males and females, 4) decreased albumin concentration in males, and 5) decreased globulin concentration in females. At the 40 mg/kg bw/day dose, the following changes were considered treatment-related: 1) decreased phospholipid concentration in males, and 2) decreased globulin concentration in females. At the 20 mg/kg bw/day dose level, the following changes were considered treatment-related: 1) decreased phospholipid concentration in males, and 2) decreased globulin concentration in females.

The following test article-related macroscopic observations were noted in the 400 mg/kg bw/day dose level: enlarged livers in three males, increased incidence of foci in the lungs of both sexes, and reduced thymus size in three males and five females. No other treatment related effects were noted for all of the other parameters measured.

In the toxicokinetic portion of the study, higher CBPI plasma concentrations were observed in females as compared to males. Systemic exposure was less than dose proportional.

Based on the biochemical and hepatocellular changes at all dose levels, a NOEL (no-observed-effect level)/NOAEL (no-observed-adverse-effect Level) could not be established. The LOEL (lowest-observed-effect level)/LOAEL (lowest-observed-adverse-effect level) was 20 mg/kg bw/day, the lowest dose tested.

(2) Study 2

Title: 13-Week Oral (Gavage) Toxicity Study in the Wistar Rat with a 4-Week Recovery Period with Carbamoyl Benzamide Phenyl Isoxazoline (CBPI). (Study No. D47345)

Report Date: May 24, 2013

Study Location: Itingen, Switzerland

Study Design: In this oral toxicity study (GLP-compliant and following OECD Test Guideline 408), CBPI (purity 99.6%) was administered daily via gavage to Wistar rats at dose levels of 2, 4, or 8 mg/kg bw/day for a period of 13 weeks (10 rats/sex/group). A control group was treated similarly with the vehicle only (0.5% carboxymethylcellulose (w/v) aqueous solution containing 0.1% polysorbate 80 (v/v)). An additional group was treated for 13 weeks and then allowed a 4-week recovery period (5 rats/sex). A satellite group were used for toxicokinetic/exposure evaluations (9 rats/sex/group and 3 rats in the control group). Detailed cage-side observations were recorded at least once daily. Clinical observations, food consumption, and body weights were recorded weekly. FOB including measurement of grip strength and locomotor activity and ophthalmological examinations were performed. Estrus stage was determined for all females. Hematology, clinical biochemistry analyses, and urinalyses measurements were conducted. Macroscopic examinations and organ weights were recorded. Histological examinations were performed on organs, tissues, and lesions.

Results and Conclusion: No CBPI-related mortalities were observed during the study. One male and one female in the control group, one male in the low dose group, and one female in the mid-dose group were found dead during the treatment period. The deaths were determined to be caused by the accidental aspiration of the dose formulation in the airways, which caused lesions in the respiratory tract including lung hemorrhages.

In the toxicokinetic portion of the study, higher CBPI plasma concentration was observed in females than males. Systemic exposure was less than dose proportional. Accumulation was observed only during the first 4 weeks of treatment and thereafter it remained unchanged for the remaining period of the study.

It was concluded that there were no treatment-related effects up to the highest dose tested (8 mg/kg bw/day).

b. Subchronic Oral Toxicity Study in Non-Rodents

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog. (Study No. D47334)

Report Date: March 20, 2015

Study Location (in-life): Itingen, Switzerland

Study Design: This GLP study was conducted according to OECD Test Guideline 409 and Veterinary International Conference on Harmonization (VICH) GL 31 with minor deviations that would not significantly affect the results. The purpose of this study was to determine the toxicity of repeated dose CBPI administration to beagle dogs for 13 weeks. Four groups of beagle dogs (4 animals/sex/group) were orally administered daily by capsule the test article, CBPI (purity 99.9%), at 0, 2, 4, and 8 mg/kg bw/day. The control group contained empty gelatin capsules. General observations and clinical observations were performed. Body weight and food consumption were recorded. Ophthalmological examinations, hematology, clinical chemistry, and urinalysis measures were performed. At termination of the study, necropsy examinations were performed, and selected organs were weighed. Macroscopic and histopathological evaluations were conducted. Plasma was collected for CBPI concentration determination and toxicokinetic analysis.

Results and Conclusion: All animals survived until termination of the study. No treatment related clinical observations were reported. No changes in food consumption or body weight were observed. No treatment related effects on ophthalmological endpoints, hematology, urinalysis or macroscopic findings were noted. Cholesterol, triglycerides, and phospholipids were reduced at 4 and 8 mg/kg bw/day for both males and females. Sex-related differences in toxicokinetic parameters were not observed. No organ weight changes were noted. No macroscopic or histopathological findings were considered treatment related.

A NOEL/NOAEL of 2 mg/kg bw/day was established based on reductions in cholesterol, triglycerides, and phospholipids at the next two highest doses.

c. Chronic Oral Toxicity Study in Non-Rodents

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): 52-Week Oral (Capsule) Toxicity Study in the Beagle Dog. (Study No. D84111)

Report Date: August 18, 2015

Study Location (in-life): Itingen, Switzerland

Study Design: This GLP study was conducted according to OECD Test Guideline 452 and VICH GL 37. The purpose of this study was to determine the toxicity of repeated dose CBPI administration to beagle dogs for 52 weeks. Four groups of beagle dogs (4 animals/sex/group) were orally administered daily by capsule the test article, CBPI (purity 99.9%), at 0, 1, 2, and 4 mg/kg bw/day. The control group contained empty gelatin capsules. General observations and clinical observations were performed. Body weights and food consumption were recorded. Ophthalmological examinations, hematology, clinical chemistry, and urinalysis measures were performed. At termination of the study, necropsy examinations were performed, and selected organs were weighed. Macroscopic and histopathological evaluations were conducted. Plasma was collected for CBPI concentration determination and toxicokinetic analysis.

Results and Conclusion: All animals survived until termination of the study. No treatment related clinical observations were reported. No changes in food consumption or body weight were observed. No treatment related effects on ophthalmological endpoints, hematology, or macroscopic findings were noted. A reduction in urine osmolality was reported for females at 4 mg/kg bw/day. Cholesterol, triglycerides, and phospholipids were reduced at 2 and 4 mg/kg bw/day for males and 4 mg/kg bw/day for females. Sex-related differences in toxicokinetic parameters were not observed. Plasma CPBI concentrations demonstrated dose proportionality was observed between 1 and 4 mg/kg bw/day on week 1 but was below proportionality on weeks 26 and 52. No treatment related organ weight changes were noted. No macroscopic or histopathological findings were considered treatment related.

A NOEL/NOAEL of 1 mg/kg bw/day was established based on reductions in cholesterol, triglycerides, and phospholipids in males at the next two higher doses.

d. Oral Developmental Toxicity Study in Rodents

Title: Prenatal Developmental Toxicity Study in the Han Wistar Rat. (Study No. C45228)

Report Date: February 3, 2012

Study Location (in-life): Füllinsdorf, Switzerland

Study Design: This GLP study was conducted according to OECD Test Guideline 452 and VICH GL32. This study was designed to evaluate the toxicity of CBPI on the pregnant rat and on the developing fetus following repeated oral exposure. Four groups of female Han Wistar rats positive for presence of sperm or copulatory plug (22 rats/group) were daily gavaged with CBPI (purity 99.6%) at dose levels of 0, 100, 300, or 1000 mg/kg bw/day (10 mL/kg bw) from day 5 to 20 post coitus. Control rats were administered the vehicle only (0.5% carboxymethylcellulose (w/v) in aqueous solution containing 0.1% polysorbate 80 (v/v)).

Mortality, clinical signs, food consumption, and body weight were recorded during the study. All females were sacrificed on day 21 post coitus and the fetuses were removed by Caesarean section. Postmortem examination included examination of all internal organs with emphasis on the uterus, uterine contents, position of fetuses in the uterus and the number of corpora luteum on the ovaries. The uterus with the fetuses was weighed to determine the corrected body weight gain. The individual fetuses were sexed, weighed, and examined for gross external abnormalities. Half of the fetuses collected were allocated for visceral organ evaluation while the other half were used to assess skeletal malformation and developmental variations.

Results and Conclusions: At 300 and 1000 mg/kg bw/day, food consumption was reduced leading to corresponding reductions of mean body weight, body weight gain, and corrected body weight gain in dams in a dose-dependent manner. The incidences of dilated renal pelvis and ureter as well as the incidence of interrupted costal cartilage in the fetuses were dose-dependently increased. In addition, incidences of supernumerary ribs per fetus and per litter basis were increased. At 100 mg/kg bw/day, no test article-related changes were observed. The dose level of 100 mg/kg bw/day was determined to be the maternal and developmental NOEL/NOAEL.

e. Oral Developmental Toxicity Study in Non-Rodents

(1) Study 1

Title: Oral Prenatal Developmental Toxicity Study in the Himalayan Rabbit. (Study No. D06081)

Report Date: December 20, 2013

Study Location (in-life): Füllinsdorf, Switzerland

Study Design: This GLP study followed OECD Test Guideline 414 and was designed to evaluate the toxicological effects of CBPI on maternal outcomes as well as on the developing fetuses. Four groups of pregnant Himalayan (CrL:Chbb) rabbits (20 per treatment group) were daily gavaged with CBPI (purity 99.7%) at dose levels of 0, 50, 250, or 1000 mg/kg bw/day (4 mL/kg bw) from days 6 to 27 post coitus. Control rabbits were administered the vehicle only (0.5% carboxymethylcellulose (w/v) in aqueous solution containing 0.1% polysorbate 80 (v/v)). In addition, three rabbits per treatment group were included for toxicokinetic evaluation.

Mortality, clinical signs, food consumption, and body weight were measured. All rabbits were sacrificed on day 28 post coitus, and the uteri with the fetuses were removed. Postmortem examination included macroscopic examination of all internal organs of the dam, corpora lutea count, uterus, uterine contents, and position of fetuses in the uterus. Fetuses were weighed individually, sexed and examined for gross external abnormalities, visceral and skeletal malformations, and developmental variations. A toxicokinetic analysis was performed on plasma CBPI on day 6 and day 27 post coitus to evaluate for bioaccumulation.

Results and Conclusions: All dose levels reduced food consumption in a dose-dependent manner in the dams. At 1000 mg/kg bw/day, reduced food consumption resulted in a corresponding reduction in body weight gain in dams and fetal body weight loss. In addition, increased post-implantation loss correlated with reduced number of fetuses per dam.

At 250 and 1000 mg/kg bw/day, fetal body weights were reduced in a dose-dependent manner. There were increased incidences of severely distended urinary bladder, which was associated with unilateral or bilateral dilatation of the renal pelvis and unilateral dilation of the ureter. In addition, urinary tract variation was observed.

At 50, 250, and 1000 mg/kg bw/day, severe unilateral and bilateral fusion between the body and arches of cervical vertebrae #2 to #5, especially cervical vertebrae #2 were observed in several litters. At 250 and 1000 mg/kg bw/day, increased incidences of non-ossification and incomplete ossification of the limbs and sternebrae #5 was observed. Systemic exposure was less than dose proportional, however, accumulation occurred with repeat dosing, with day 27 post coitus exposure being 2.5 fold to 4.8 fold higher than day one.

Maternal NOEL/NOAEL was established at 50 mg/kg bw/day. While a NOEL/NOAEL for developmental effects could not be established, a LOEL/LOAEL for developmental effects was established at 50 mg/kg bw/day, the lowest dose tested.

(2) Study 2

Title: Oral Prenatal Developmental Toxicity Study in the Himalayan Rabbit. (Study No. D52036)

Report Date: January 17, 2014

Study Location (in-life): Füllinsdorf, Switzerland

Study Design: This GLP study was conducted following OECD Test Guideline 414. Four groups of 20 pregnant Himalayan (Crl:Chbb) rabbits were treated with CBPI (purity 99%) by daily gavage at doses of 0, 10, 25, or 250 mg/kg bw/day (4 mL/kg bw) from days 6 to 27 post coitus. Control animals were treated with the vehicle alone. A satellite toxicokinetic group (5 females/ group) was also included in the study from which blood samples were collected on days 6 and 27 post coitus.

Mortality, clinical signs, food consumption, body weight, hematology, clinical biochemistry, and clinical pathology on days 12, 20, and 28 post coitus were measured. On day 28 post coitus, the uteri and contents with fetuses were weighed. Following postmortem examinations, the organ weight, liver histopathology, corpora lutea count, uterus, uterine contents and position of fetuses in the uterus were evaluated. Fetuses were removed from the uterus, and blood samples were taken. Fetuses were then weighed individually, and examined for external, visceral, and skeletal abnormalities. Blood from each litter was pooled. The urinary tract of the fetus was evaluated by histopathological examination.

Results and Conclusions:

Maternal Outcomes: At 25 and 250 mg/kg bw/day, food consumption was reduced in a dose-dependent manner. However, this did not impact body weight, body weight gain or corrected body weight gain.

At 250 mg/kg bw/day, three rabbits were not pregnant and fetus resorption was noted in one pregnant rabbit. One rabbit per group including the control group was not pregnant. The mean number of implantation sites, post-implantation loss, and mean number of fetuses per dam were similar at all dose levels.

Clinical pathological changes identified in the 250 mg/kg bw/day dose group until 28 days post coitus included increased numbers of leukocytes and lymphocytes, reduced concentration of β-1-globulin and reduced plasma cholinesterase activities. In addition, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase and glutamate dehydrogenase were increased at several sampled time points. Further, urea, blood urea nitrogen (BUN), triglycerides, phospholipids and cholesterol were reduced. The concentrations of α-2-globlin and γ-globulin and calcium were also reduced.

At 25 mg/kg bw/day, reduced concentrations of triglycerides, phospholipids, and cholesterol as well as reduced activity of plasma cholinesterase were observed. In addition, reduced concentrations of urea and BUN were noted on day 20. Other effects included reduced concentrations of α-2-globulin and γ-globulin. At 10 mg/kg bw/day, reductions in concentrations of triglycerides, phospholipids, cholesterol, and cholinesterase on days 12, 20, and 28, with slight recovery on day 28 post coitus when compared to control values was observed.

The incidence and severity of fatty liver change was increased at all dose levels. CBPI induced microvesicular to macrovesicular fatty liver change, which was distributed periportal to centrilobular. In addition, minimal hepatocellular hypertrophy was noted in one female at 25 mg/kg bw/day and three females at 250 mg/kg bw/day dose group. Fatty liver change was also noted in control animals however, it was considered a normal physiological response because it was of lower severity and located predominately periportal.

Systemic exposure to CBPI was achieved and resulted in less than dose proportionality. Accumulation was observed with repeat dosing and the exposure achieved on day 27 was 2.3 to 6.0 fold higher than what was recorded on day 6 post coitus.

Fetal Outcomes: At 250 mg/kg bw/day, mean fetal body weight was reduced on a litter and fetal basis. In addition, increased incidences of distended urinary bladder associated with dilatation of ureter and renal pelvis were observed. Histopathological findings showed functional distension of the urinary bladder and minimal to moderate back flow pelvic dilation along with minimal hydronephrosis of 6 fetuses (1 litter).

At 250 mg/kg bw/day, severe unilateral or bilateral fusion between the body and arches of cervical vertebrae #2 were noted in 3 litters. Non-severe unilateral or bilateral fusions in cervical vertebrae #2 were noted in 9 litters. A reduced incidence of increased ossification of the femur occurred at 250 mg/kg bw/day. At 25 mg/kg bw/day, non-severe unilateral fusion in cervical vertebrae #2 was noted in 2 litters.

Because the test article-treatment related maternal findings were observed at all dose levels, a NOEL/NOAEL for maternal toxicity could not be established, however, a LOEL/LOAEL of 10 mg/kg bw/day was established. Since fetal toxicity was evident at >10 mg/kg bw/day, the oral NOEL/NOAEL for developmental toxicity was established at 10 mg/kg bw/day.

f. Oral Reproduction Toxicity Studies in Rats

(1) One-Generation Reproduction Toxicity Study

Title: Oral One-Generation Reproduction Toxicity Study in the Han Wistar Rat. (Study No. D06103)

Report Date: February 3, 2014

Study Location (in-life): Füllinsdorf, Switzerland

Study Design: This GLP study was conducted according to OECD Test Guideline 415 to evaluate the cumulative oral toxicity of CBPI on reproductive function and development. Four groups of RccHan: WIST rats (24 rats/ sex), eight weeks of age, were treated with CBPI (purity 99.7%) at dose levels of 0, 50, 100 or 500 mg/kg bw/day. Males and females were administered the test article or vehicle (control group) from day one of the pre-mating period for 70 days and during mating for 21 days. Females continued to be treated during gestation and lactation in each phase for 21 days. Males continued to be treated until the day before sacrifice. During mating, each individual female was housed with one male until evidence of copulation. Weekly food consumption, daily body weights, and clinical observations were recorded during the study. Reproductive performance of the parents was evaluated. In the offspring, neonatal morbidity, mortality, growth, and development were assessed.

All females were sacrificed at or shortly after weaning whereas males were sacrificed after necropsy of females. All female rats were examined macroscopically, and implantation sites were counted. All excised organs were collected and weighed, and the reproductive organs, liver, kidneys, thymus, adrenal glands and spleen were processed for histological examination. The ovaries of the control and high dose groups were examined, and evaluated for primordial, growing and antral follicles. Corpora lutea were counted on one section per ovary. In males, semen analysis and spermatid count were performed, which included examination of sperm motility, morphology, spermatid and sperm count.

Results and Conclusions:

Parental Generation: At 500 mg/kg bw/day in females, food consumption was reduced during the pre-mating, gestation and lactation periods, and absolute body weight was reduced at the end of the pre-mating and during the gestation periods. The implantation rate was reduced and post-implantation loss was increased, resulting in a lower mean number of pups at the first litter check. In addition, increased pup breeding loss was observed during days 5 to 21 post-partum at 500 mg/kg bw/day that contributed to an overall reduction on weaning index or survival index.

The liver to body weight ratio was increased in the 500 mg/kg bw/day dose group. In all CBPI-treated groups an increased incidence of enlarged liver, tan discoloration, and accentuated lobulation were observed. These gross findings in the liver correlated microscopically with vacuolation in the cytoplasm of the hepatocytes. The lung to body weight ratio was increased in the 500 mg/kg bw/day dose group. In all CBPI-treated groups, macroscopically the lungs showed an increased incidence of foci. This correlated microscopically with alveolar histiocytosis, alveolar bronchiolization and foreign material in the bronchial-alveolar lumen. Changes were more pronounced in females than in males. Rats from the 500 and 100 mg/kg bw/day dose groups showed an increase in the absolute and relative adrenal weight ratio. Macroscopically, an increased incidence of discoloration of the adrenal glands was observed, which was correlated microscopically with cytoplasmic vacuolation of the adrenal gland cortices in both sexes. Females at 500 mg/kg bw/day showed hypertrophy of the adrenal gland cortex. CBPI treatment at 500 mg/kg bw/day dose levels resulted in reductions of the absolute and relative thymus weight ratios that correlate microscopically with lymphoid atrophy.

F1 Offspring: In pups produced from dams administered 500 mg/kg bw/day, their body weights were reduced from postnatal days (PNDs) 7 to 14 but recovered from PNDs 14 to 21. However, comparing body weight gain from PNDs 1 to 21, the gain was reduced at 100 and 500 mg/kg bw/day.

The absolute brain weight was decreased in a dose-dependent manner at all dose levels for both male and female pups. In addition, at 500 mg/kg bw/day, the brain to body weight ratio was increased in males. Likewise, in male and female pups, thymus to body weight ratios was reduced in a dose-dependent manner. The absolute and relative spleen weight was also reduced at the highest dose level. In male and female pups, lymphoid atrophy was observed in the thymus at all dose levels and the spleen of the medium and high dose level groups.

A NOEL/NOAEL for maternal toxicity could not be established because of significant increases in enlarged liver (absolute and relative) and increased lobulation of the liver corresponding with an increase in cellular vacuolation consistent with fat accumulation in the cytoplasm of the hepatocytes and adrenal gland weight (relative and absolute) at all dose levels. A maternal toxicity LOEL/LOAEL was established at 50 mg/kg bw/day. In addition, a NOEL/NOAEL could not be established for developmental toxicity because of lymphoid atrophy of the thymus tissue, and reduced brain and thymus weight at the lowest dose level tested. A LOEL/LOAEL was established at 50 mg/kg bw/day for developmental toxicity. The NOEL/NOAEL for reproduction toxicity was established at 100 mg/kg bw/day due to the lower number of live fetuses at first litter check and higher fetal loss during the lactation period at 500 mg/kg bw/day.

(2) Two-Generation Oral Reproduction Toxicity Study

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): Two-Generation Reproduction Toxicity Study in the Han Wistar Rat. (Study No. D69698)

Report Date: September 14, 2015

Study Location: Itingen, Switzerland

Study Design: This GLP study was conducted following the procedures in OECD Test Guideline 416 and was designed to evaluate the effects of CBPI on the reproductive processes in rats. Four randomized groups of Wistar rats (the F0 generation; 24/sex/group) were administered the test article, CBPI (purity 99.9%), daily by gavage at 0, 8, 50, or 500 mg/kg bw/day for 10 weeks before mating, and the daily treatment continued through the mating period (up to two weeks), gestation, parturition, lactation, until termination following the weaning of the offspring. Upon parturition, the pups (the F1 generation) were evaluated; the litter were culled to 8 (sex-balanced if possible) on PND 4, and remaining pups nursed to weaning on PND 21. One male and one female from each F1 litter were randomly selected to continue treatment for 10 weeks before breeding to produce the F2 offspring. An additional male and female from each F1 litter of all doses continued treatment for FOB test evaluation.

Daily cage-side observations, weekly clinical examinations, weekly food consumption, daily measurement for body weight, daily recording of mortality or missing pups, behavioral and physical abnormalities of live pups, and the sex ratio of litters were all recorded. Physical development of the offspring was evaluated in terms of the ages of pinna unfolding, eye opening, and lower incisor and coat development. The age and body weight at which vaginal opening and preputial separation, as appropriate for the sex, were recorded for the F1 animals. Standard FOB tests were performed in the F1 generation on PND 42.

All parental animals were necropsied and examined macroscopically, with a set of organs and tissues sampled and preserved; females were examined for implantation sites. Excess animals and pups were subjected to macroscopic examination. Organ weights were collected at necropsies and processed for microscopic evaluation, histology and standard sperm analyses.

Results and Conclusions: For the parental animals of both F0 and F1 generations, in addition to the unscheduled death that occurred in the high-dose group, reductions in food consumption and body weight gain at the high dose, especially during the premating stage, were evident. Abdominal distention appeared to be a prominent effect at the high-dose, supported by both clinical observation and necropsy findings. Organ weight changes, both in absolute and relative terms, were observed for thymus, adrenals, liver, and lungs in the high dose group and correlated with histological findings. Fatty changes in the liver were observed in both F0 and F1 animals, at the mid- and high-dose groups. Increased implantation losses and reduced litter size were seen in the high dose group of both breeding cycles. Impaired growth and delayed functional maturation were also seen in the F1 animals in the high-dose group.

Increased adrenal weight in the F0 males, increased liver weight with the emergence of fatty changes in both F0 and F1 animals, increased food consumption and body weight of the F1 females during the premating and gestation periods, and the hypertrophy of the zona fasciculata in the adrenals of F1 animals were all reported in the mid-dose group. In the low-dose group only a few histological findings were observed: cortical vacuolation in the adrenal glands and inflammatory reactions in the lung tissue.

It was concluded that the NOEL/NOAEL for general toxicity was 8 mg/kg bw/day; and the NOEL/NOAEL for the reproductive toxicity was 50 mg/kg bw/day.

g. Genetic Toxicity Studies

The findings from the genotoxicity testing are presented in Table IV.1. and described in detail below. Results from the genetic toxicology studies support the conclusion that fluralaner is not a genotoxic compound.

Table IV.1. Summary of Fluralaner Genotoxicity Studies

Study Type	Study No.	Results
Bacterial Reverse Mutation Assay (Ames Test)	CCR 1289101	Negative
In Vitro Mammalian Cell Gene Mutation Test	CCR 1289102	Negative
In Vitro Mammalian Chromosome Aberrations Test	CCR 1289103	Negative

Study Type	Study No.	Results
In Vivo Micronucleus Test	CCR 1289104	Negative

(1) Bacterial Reverse Mutation Assay (Ames Test)

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay. (Study No. CCR 1289101)

Report Date: July 19, 2010

Study Location: Rossdorf, Germany

Study Design: This study followed the OECD Test Guideline 471 (1997) and was OECD GLP-compliant. The bacteria test strains included *Salmonella typhimurium* TA1535, TA1537, TA98, and TA100, and *Escherichia coli* WP_2 *uvrA* (pKM101). The assay was performed in two independent experiments (I and II), both with and without liver S9 fraction for metabolic activation. Experiment I used the direct plate incorporation method, and Experiment II used the pre-incubation method. The test concentrations of CBPI (purity 99.6%, in DMSO solvent) for each experiment are: 0, 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate.

For assays without metabolic activation, the following positive controls were utilized: sodium azide (NaN_3) for TA1535 and TA100, 4-nitro-*o*-phenylenediamine for TA1537 and TA98, and methyl methane sulfonate (MMS) for WP_2 *uvrA* (pKM 101). For assays with metabolic activation, 2-aminoanthracene was used as the positive control.

Results and Conclusion: The bacteria plates treated with CBPI showed normal background growth at all concentrations tested with and without metabolic activation in both experiments. No increase in revertant colony numbers of any of the test strains was observed, either in the presence or absence of metabolic activation. All positive control plates showed expected increases in the number of revertant colonies. It was concluded that CBPI was not a mutagen under the conditions tested.

(2) *In Vitro* Mammalian Cell Gene Mutation Test

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): Cell Mutation Assay at the Thymidine Kinase Locus ($TK^{+/-}$) in Mouse Lymphoma L5178Y Cells. (Study No. CCR 1289102)

Report Date: April 4, 2012

Study Location: Rossdorf, Germany

Study Design: This study followed the OECD Test Guideline 476 (1997) and was OECD GLP compliant. It was conducted to test the potential of CBPI to induce mutations at the thymidine kinase locus in the mouse lymphoma cell line L5178Y. The assay comprised two independent experiments.

The first experiment was performed with CBPI (99.6% purity) for a treatment period of 4 hours with and without metabolic activation. The second experiment included a treatment period of 4 hours with and without metabolic activation and an additional 24-hour treatment period without metabolic activation. DMSO was used as the solvent for CBPI as well as the vehicle control; MMS was used as positive control for experiments without S9 fraction, and cyclophosphamide (CPA) was used as positive control for experiments with S9 fraction.

A range-finding pre-experiment revealed strong cytotoxic effects caused by CBPI treatment. The tests were conducted in two parallel cultures (Cultures I and II). At least four concentrations of CBPI were selected for mutational analyses for each culture, based on cytotoxicity. After 4 hours or 24 hours of treatment, CBPI and controls were removed, and the cells were incubated for an expression and growth period of 48 hours to calculate relative suspension growth (RSG). Subsequently, the cells from each treatment group were seeded into microplates in the culture media containing trifluorothymidine (TFT). Cloning efficiency was determined by comparison to viability in media without TFT. The number of colonies in the plates were then counted.

Results and Conclusion: The mutation frequency of certain treatment groups was increased above the control values; however, the cytotoxicity was so severe (RSG at 5.4 and 0.7%) in those cases that such results may not be reliable. Overall, there was no consistent and dose-dependent increase in mutant colony numbers. There was also no relevant shift of the ratio of small versus large colonies at up to the maximum concentration of the test item. Therefore, the observed increases in mutation frequency in those few treatments were not sufficient to consider CBPI mutagenic in mammalian cells.

It was concluded that CBPI did not induce consistent mutations in the assay either in the absence or presence of metabolic activation.

(3) *In Vitro* Mammalian Chromosome Aberrations Test

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): Chromosome Aberration Test in Human Lymphocytes *in vitro*. (Study No. CR 1289103)

Report Date: February 16, 2012

Study Location: Rossdorf, Germany

Study Design: The test article, CBPI (purity 99.6%), was assessed for its potential to induce chromosomal aberrations in human lymphocytes *in vitro*. This study followed the OECD Test Guideline 473 (1997) and was OECD GLP compliant. Four independent experiments: Experiment IA, IB, IIA, and IIB; Experiments IB and IIB were partial repeats of Experiments IA and IIA, respectively.

The range of CBPI concentration in the experiments was between 0.2 to 100 µg/mL. DMSO was used as the CBPI solvent and also as the negative control; ethyl methanesulfonate (EMS) was used as the positive control for experiments without metabolic activation, and cyclophosphamide (CPA) was used as the positive control for experiments with metabolic activation.

The cells used in this study were human lymphocytes in whole blood samples from four adult females. Cytotoxicity was assessed based on the percentages of mitotic suppression in comparison to the negative control by counting 1000 cells per culture. At each dose two parallel cultures were treated with CBPI and the controls for 4, 22, or 46 hours. Cells were harvested and chromosomal preparation was carried out after 22 hours (Exp. IA and IB) and 46 hours (Exp. IIA and IIB). For each culture 100 metaphases were scored for structural chromosomal aberrations, except for the positive controls in Experiment IIB in the absence of S9 mix, of which only 50 metaphases were scored. Metaphase cells were analyzed microscopically. Breaks, fragments, deletions, exchanges and chromosomal disintegrations were recorded as structural chromosome aberrations. Gaps were also documented. A total of 100 well-spread metaphases were recorded per culture.

Results and Conclusion: Results of the study suggest that there were some inconsistences for CBPI concentration-dependent cytotoxicity across the experiments (due to unsatisfactory cytotoxicity data, Experiment IA and IIA were repeated as Experiments IB and IIB). Based on cytotoxicity observations, only a subset of cells were processed for chromosomal aberration analyses.

Some high doses of CBPI in the four experiments that were analyzed for chromosomal aberration did not result in expected cytotoxicity; the high dose was expected to yield around 50% or lower cytotoxicity. The negative control and positive controls produced effects as expected, and the results were consistent with historical controls.

Overall, the data suggest that CBPI did not cause chromosomal aberrations *in vitro* under the experimental conditions. Therefore, CBPI was considered to be non-clastogenic under the conditions tested.

(4) *In Vivo* Micronucleus Test

Title: Carbamoyl Benzamide Phenyl Isoxazoline (CBPI): Micronucleus Assay in Bone Marrow Cells. (Study No. CCR 1289104)

Report Date: September 19, 2012

Study Location: Rossdorf, Germany

Study Design: This study was performed to investigate the potential of CBPI to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The study followed OECD Test Guideline 474 (1997), and the study was OECD GLP compliant.

CBPI (purity 99.6%) was given to NMRI mice by gavage at three dose levels: 500, 1000, and 2000 mg/kg bw (10 mL/kg bw). The test article was formulated in 0.5% carboxymethylcellulose (w/v) aqueous solution containing 0.1% polysorbate-80 (v/v) and was also used as vehicle control. CPA was used as the positive control. A total of 72 mice were divided into six treatment groups. All treated animals were given a single oral administration. At 24 or 48 hours post treatment, bone marrow samples were obtained from the femurs.

A total of 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. The cytotoxic effect of CBPI, indicated by the ratio between polychromatic and total number of erythrocytes, was determined for each sample and reported as the number of PCEs per 2000 erythrocytes.

Results and Conclusion: There were no significant changes in the ratio of PCE to total number of erythrocytes after treatment with CBPI. There was no significant increase in the frequency of micronuclei at any dose level or sampling interval after administration of the CBPI. The positive control caused a substantial increase of induced micronucleus frequency. The results of the vehicle and positive controls were in agreement with historical control data.

It was concluded that CBPI did not induce micronuclei in bone marrow cells of the mouse and was considered to be non-mutagenic in the micronucleus assay.

2. Point of Departure for the Toxicological Acceptable Daily Intake (ADI) Determination

Studies considered for the determination of the point of departure for chronic exposure to total residues of fluralaner are summarized in Table IV.2. Based on these toxicology studies, the NOEL/NOAEL of 1 mg/kg bw/day from the chronic (52-week) oral toxicity study in dogs (Study No. D84111) was determined to be the most appropriate for the derivation of the toxicological ADI for fluralaner.

Table IV.2. Summary of NOEL/NOAEL or LOEL/LOAEL in Toxicology Studies for Fluralaner

Study Type	Study No.	NOEL/NOAEL or LOEL/LOAEL
Subchronic Oral Toxicity Study in Rodents	D47345	NOEL/NOAEL 8 mg/kg bw/day (highest dose tested)
Subchronic Oral Toxicity Study in Non-Rodents	D47334	NOEL/NOAEL 2 mg/kg bw/day
Chronic Oral Toxicity in Non-Rodents	D84111	NOEL/NOAEL 1 mg/kg bw/day
Oral Developmental Toxicity Study in Rodents	C45228	Maternal and Developmental toxicity: NOEL/NOAEL 100 mg/kg bw/day
Oral Developmental Toxicity Study in Non-Rodents	D52036	Maternal toxicity: LOEL/LOAEL 10 mg/kg bw/day Developmental toxicity: NOEL/NOAEL 10 mg/kg bw/day
Two-Generation Oral Reproductive Toxicity Study in Rats	D69698	General toxicity: NOEL/NOAEL 8 mg/kg bw/day Reproductive toxicity: NOEL/NOAEL 50 mg/kg bw/day

3. Toxicological ADI

The toxicological ADI for total residue of fluralaner is calculated using the following formula. The NOEL/NOAEL of 1 mg/kg bw/day from the chronic oral dog toxicity study was selected. A safety factor of 100 was applied to account for a 10-fold factor for animal-to-human variability and a 10-fold factor for human-to-human variability in sensitivity.

$$\text{Toxicological ADI} = \frac{\text{NOEL/NOAEL}}{\text{Safety Factor}} = \frac{1\ \text{mg/kg bw/day}}{100}$$

$$= 0.01\ \text{mg/kg bw/day or } 10\ \mu\text{g/kg bw/day}$$

The toxicological ADI for total residue of fluralaner is established at 10 µg/kg bw/day.

C. Establishment of the Final ADI

Because fluralaner is not an antibacterial agent and has no or negligible antibacterial activity, a microbiological ADI was not needed. Therefore, the toxicological ADI (10 µg/kg bw/day) is established as the final ADI for total residue of fluralaner.

D. Safe Concentrations for Total Residues in Edible Tissues

The calculation of the tissue safe concentrations is based on the “General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals” (Guidance for Industry #3, May 2022) and reflects the partition (9% of the ADI for meat, 20% of the ADI reserved for milk, 71% of the ADI for eggs). The safe concentration for total residues of fluralaner in edible tissues of laying hens and replacement chickens is calculated using the following formula using the human body weight of 60 kg:

$$\text{Safe Concentration}=\frac{\text{Percent Partition} \times \text{ADI x Human Body Weight}}{\text{Food Consumption Value}}$$

Table IV.3. Summary Table of Safe Concentrations for Total Residues of Fluralaner in Edible Tissues of Laying Hens and Replacement Chickens

Edible Tissue		ADI Partition (%)	Daily Food Consumption Values (g)	Safe Concentration (µg/kg or ppb)
Meat	Muscle	9%	300	180
	Liver	9%	100	540
	Skin/Fat	9%	50	1080
Eggs		71%	100	4260

E. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Total Residue and Metabolism Studies

      (1) Tissue Total Residue and Metabolism Study

Title: A Pivotal Metabolism and Total Residue Depletion Study in Broiler Chickens Following Oral Administration of [^{14}C]-Fluralaner in Water on Two Occasions, Seven Days Apart. (Study No. S18221-00)

Study Dates: May 03, 2019 to November 02, 2021

Study Location: Tranent, East Lothian, United Kingdom

Study Design:

Objective: The objective of this study was to determine concentrations of total residues of fluralaner and characterize fluralaner metabolites in chicken muscle, liver, skin with fat in natural proportions (skin/fat), and kidney tissues following oral administration of fluralaner at a nominal dose of 0.66 mg fluralaner/kg bw on two occasions spaced seven days apart.

Study Animals: Seventy-eight Ross 308 broiler chickens (39 males and 39 females) were used in this study. Chickens were 21 days of age and weighed 775.35 ± 110.93 g at the start of the study.

Experimental Design: The study was conducted in accordance with Good Laboratory Practices (GLPs; 21 CFR § 58). Chickens were assigned to one of six treatment groups or one spare treated group (Table IV.4.).

Table IV.4. Experimental Groups

Group	Number of Chickens	Assigned Withdrawal Period (days)
1	6 males and 6 females	0.5
2	6 males and 6 females	1
3	6 males and 6 females	2
4	6 males and 6 females	4
5	6 males and 6 females	8
6	6 males and 6 females	12
7 (spare chickens)	3 males and 3 females	As needed

Drug Administration: Chickens were dosed by oral gavage with a solution containing a mixture of [^{14}C]-fluralaner and fluralaner on two occasions spaced seven days apart. On each dosing occasion, the total nominal dose (0.66 mg fluralaner/kg bw) was split into two oral gavages administered 12 hours apart.

Measurements and Observations: At the assigned withdrawal period (Table IV.4.), chickens were slaughtered by cervical dislocation followed by exsanguination. Samples of muscle, liver, skin/fat, and kidney were collected from each chicken.

Concentrations of total residues of fluralaner were determined in individual samples by combustion followed by liquid scintillation counting.

Three tissue samples *per* withdrawal period and *per* sex were pooled, extracted, and subjected to metabolite profiling by high-pressure liquid chromatography coupled with a radioactivity detector (radio-HPLC).

Parent fluralaner and two major fluralaner metabolites were isolated in liver and kidney samples by liquid chromatography and structurally elucidated by high resolution mass spectrometry.

Concentrations of parent fluralaner were determined in individual samples by liquid chromatography with mass spectrometry detection (LC-MS/MS) using a validated analytical procedure.

Results: Total residues of fluralaner (Table IV.5.) and parent fluralaner (Table IV.6.) were detected in tissue samples and depleted over time. These data enabled the relationship between parent fluralaner and total residues of fluralaner to be established (Table IV.7.). The ratio between parent fluralaner (marker residue) and total residues of fluralaner (M:T) ranged between 0.56 and 0.92 for muscle, 0.60 and 1.00 for liver, 0.36 and 0.95 for skin/fat, and 0.49 and 1.00 for kidney.

Table IV.5. Mean (± standard deviation) Concentrations (ppb) of Total Residues of Fluralaner in Tissues from Chickens Orally Administered Fluralaner at a Nominal Dose of 0.66 mg/kg bw on Two Occasions Spaced Seven Days Apart

Withdrawal Period (days)	Muscle	Liver	Skin/Fat	Kidney
0.5	317.54 ± 24.80	1953.82 ± 195.02	1483.12 ± 113.27	1184.39 ± 122.44
1	274.66 ± 27.22	1730.82 ± 192.60	1310.04 ± 175.40	1016.30 ± 177.31
2	228.77 ± 32.87	1445.95 ± 159.04	1151.32 ± 149.53	873.12 ± 111.13
4	134.55 ± 30.67	754.30 ± 167.81	724.87 ± 128.82	494.75 ± 102.17
8	53.93 ± 13.03	329.21 ± 105.81	345.86 ± 70.76	200.19 ± 57.41
12	27.43 ± 8.54	153.66 ± 68.80	183.22 ± 43.48	91.77 ± 36.35

Table IV.6. Mean (± standard deviation) Concentrations (ppb) of Parent Fluralaner in Tissues from Chickens Orally Administered Fluralaner at a Nominal Dose of 0.66 mg/kg bw on Two Occasions Spaced Seven Days Apart

Withdrawal Period (days)	Muscle	Liver	Skin/Fat	Kidney
0.5	275.07 ± 22.37	1510.87 ± 110.37	1246.34 ± 135.99	971.74 ± 110.65
1	223.23 ± 25.91	1297.53 ± 170.43	1043.75 ± 148.28	784.70 ± 128.81
2	196.42 ± 35.74	1084.73 ± 184.26	924.24 ± 148.26	651.38 ± 129.70
4	110.51 ± 31.18	597.14 ± 154.51	522.05 ± 147.71	410.05 ± 66.10
8	40.43 ± 12.29	240.52 ± 81.25	206.61 ± 56.64	<LOQ*
12	24.49 ± 5.27	120.36 ± 51.68	131.23 ± 18.03	<LOQ

*<LOQ, less than the limit of quantification (217 ppb)

Table IV.7. Ratio (range and mean ± standard deviation) between Parent Fluralaner (the marker residue) and Total Residues of Fluralaner in Tissues from Chickens Orally Administered Fluralaner at a Nominal Dose of 0.66 mg/kg bw on Two Occasions Spaced Seven Days Apart

Withdrawal Period (days)	Muscle	Liver	Skin/Fat	Kidney
0.5	Range: 0.80 to 0.92 Mean: 0.87 ± 0.04	Range: 0.71 to 0.84 Mean: 0.78 ± 0.04	Range: 0.72 to 0.94 Mean: 0.84 ± 0.06	Range: 0.72 to 0.91 Mean: 0.82 ± 0.05
1	Range: 0.65 to 0.92 Mean: 0.81 ± 0.06	Range: 0.63 to 0.81 Mean: 0.75 ± 0.05	Range: 0.69 to 0.93 Mean: 0.80 ± 0.07	Range: 0.49 to 1.00 Mean: 0.79 ± 0.18
2	Range: 0.73 to 0.92 Mean: 0.85 ± 0.05	Range: 0.64 to 0.82 Mean: 0.75 ± 0.06	Range: 0.63 to 0.95 Mean: 0.80 ± 0.10	Range: 0.61 to 0.84 Mean: 0.74 ± 0.06
4	Range: 0.66 to 0.92 Mean: 0.81 ± 0.07	Range: 0.64 to 1.00 Mean: 0.79 ± 0.13	Range: 0.36 to 0.92 Mean: 0.71 ± 0.13	Range: 0.66 to 0.80 Mean: 0.74 ± 0.04
8	Range: 0.58 to 0.82 Mean: 0.74 ± 0.07	Range: 0.63 to 0.80 Mean: 0.73 ± 0.06	Range: 0.37 to 0.85 Mean: 0.61 ± 0.15	NC*
12	Range: 0.56 to 0.87 Mean: 0.76 ± 0.10	Range: 0.60 to 0.78 Mean: 0.72 ± 0.05	Range: 0.62 to 0.77 Mean: 0.70 ± 0.06	NC

*NC, not calculable because parent fluralaner was less than the limit of quantification (217 ppb)

Parent fluralaner and two metabolites (M5 and M10) were the only major residues detected in edible chicken tissues. High-resolution mass spectrometry indicated that M5 is mono-hydroxylated fluralaner and that M10 is fluralaner carboxylic acid.

Conclusions: Liver was the tissue in which total residues of fluralaner last depleted to less than the safe concentration. This indicates that, when liver total residue concentrations are less than the liver safe concentration, total residues of fluralaner in the other edible tissues are less than their respective safe concentration. The M:T ratio in meat tissues is variable for fluralaner. When concentrations of total residues of fluralaner in liver and muscle were less than their respective safe concentrations, concentrations of parent fluralaner were approximately 320 ppb and 110 ppb, respectively. These concentrations equate to M:T ratios of approximately 0.60 and 0.61 for liver and muscle, respectively. Two major metabolites of fluralaner were detected in edible chicken tissues: M5, mono-hydroxylated fluralaner and M10, fluralaner carboxylic acid.

The data from this study indicate liver and parent fluralaner are an appropriate target tissue and marker residue, respectively, for monitoring the human food safety of edible meat tissues obtained from laying hens and replacement chickens treated with fluralaner. The data also indicate that a liver tolerance of 320 ppb and a muscle tolerance of 110 ppb are protective of human health.

(2) Egg Total Residue and Metabolism Study

Title: A Pivotal Metabolism and Total Residue Depletion Study in Laying Hens Following Oral Administration of [^{14}C]-Fluralaner in Water on Two Occasions, Seven Days Apart. (Study No. S18222-00-HID-STA-PO)

Study Dates: November 29, 2019 to November 01, 2021

Study Location: Tranent, East Lothian, United Kingdom

Study Design:

Objective: The objective of this study was to determine concentrations of total residues of fluralaner and characterize fluralaner metabolites in eggs from chickens orally administered fluralaner at a nominal dose of 0.66 mg fluralaner/kg bw on two occasions spaced seven days apart.

Study Animals: Twenty Shaver Brown laying hens were used in this study. Chickens were 34 weeks of age and weighed 1962.70 ± 84.90 g at the start of the study.

Experimental Design: The study was conducted in accordance with GLPs (21 CFR § 58).

Drug Administration: Hens were dosed by oral gavage with a solution containing a mixture of [^{14}C]-fluralaner and fluralaner on two occasions spaced seven days apart. On each dosing occasion, the total nominal dose (0.66 mg fluralaner/kg bw) was split into two oral gavages administered 12 hours apart.

Measurements and Observations: Eggs were collected twice daily during the morning and evening. The first egg collection occurred immediately prior to the first dose administration (Study Day 1), and the last egg collection occurred on Study Day 22.

Concentrations of total residues of fluralaner were determined in individual samples by combustion followed by liquid scintillation counting.

Eggs from 10 hens were selected for metabolite profiling. The eggs were selected from the two timepoints with the greatest total residue concentrations after the first dose (Study Days 7 and 8) and three timepoints with the greatest total residue concentrations after the second dose (Study Days 13, 14, and 15). Egg samples were extracted individually and subjected to metabolite profiling by radio HPLC. Parent fluralaner and one major fluralaner metabolite were isolated by liquid chromatography and structurally elucidated by high resolution mass spectrometry.

Concentrations of parent fluralaner were determined in individual samples by LC-MS/MS using a validated analytical procedure.

Results: Total residues of fluralaner (Table IV.8.) were present in eggs after the first dose was administered and increased with time. Total residues of fluralaner first peaked on Study Day 8.11 ± 0.94 with a concentration of 1016.00 ± 122.56 ppb. This was followed by a brief decline in total residues of fluralaner. Then, following the second dose, a second peak occurred at Study Day 14.11 ± 0.99 with a concentration of 1351.73 ± 178.21 ppb. After the second peak, total residue concentrations declined. Parent fluralaner (Table IV.8.) was not detectable by LC-MS/MS until Study Day 3, after which parent fluralaner concentrations in eggs followed a similar trend as total residues of fluralaner. Parent fluralaner first peaked at Study Day 7.90 ± 0.57 with a concentration of 840.18 ± 111.11 ppb. This was followed by a brief decline in parent fluralaner concentrations. Then, following the second dose, a second peak occurred at Study Day 13.90 ± 0.74 with a concentration of 1167.58 ± 166.83 ppb. After the second peak, parent fluralaner concentrations declined. These data enabled the relationship between parent fluralaner (marker residue) and total residues of fluralaner to be established (Table IV.8.). The M:T ratio between parent fluralaner and total residues of fluralaner ranged between 0.38 and 0.91.

Parent fluralaner and one metabolite (M5) were the only major residues detected in edible chicken tissues. High-resolution mass spectrometry indicated that M5 is mono-hydroxylated fluralaner.

Conclusions: Total residues of fluralaner were less than the egg safe concentration in all eggs collected. The M:T ratio in eggs is variable for fluralaner. An M:T of approximately 0.58 was determined to be appropriate for assessing the human food safety of eggs obtained from laying hens treated with Exzolt™. Based on an M:T of 0.58, when concentrations of parent fluralaner are less than 2500 ppb, total residues of fluralaner are less than the egg safe concentration. One major metabolite of fluralaner was detected in eggs: M5, mono-hydroxylated fluralaner.

Table IV.8. Total Residues of Fluralaner Concentrations, Parent Fluralaner Concentrations, and the Ratio between Parent Fluralaner and Total Residues of Fluralaner in Eggs from Laying Hens Orally Administered Fluralaner at a Nominal Dose of 0.66 mg/kg bw on Two Occasions Spaced Seven Days Apart

Study Day	Total Residues of Fluralaner (Mean ± Standard Deviation; ppb)	Parent Fluralaner (Mean ± Standard Deviation; ppb)	Ratio Range	Ratio (Mean ± Standard Deviation)
1	0.03 ± 0.07	<LOQ*	NC‡	NC
2	0.33 ± 0.25	<LOQ	NC	NC
3	71.15 ± 29.72	<LOQ	NC	NC
4	337.50 ± 87.02	354.38 ± 16.39	0.85 to 1.00	0.99 ± 0.16
5	514.07 ± 157.43	425.65 ± 100.16	0.46 to 1.00	0.99 ± 0.48
6	782.38 ± 93.95	571.67 ± 87.49	0.46 to 0.86	0.74 ± 0.14
7	943.74 ± 133.73	712.20 ± 85.66	0.67 to 0.96	0.78 ± 0.09

Study Day	Total Residues of Fluralaner (Mean ± Standard Deviation; ppb)	Parent Fluralaner (Mean ± Standard Deviation; ppb)	Ratio Range	Ratio (Mean ± Standard Deviation)
8	992.71 ± 123.66	813.24 ± 117.10	0.73 to 0.87	0.82 ± 0.04
9	954.02 ± 117.72	784.69 ± 108.49	0.79 to 0.90	0.83 ± 0.03
10	873.67 ± 118.09	713.84 ± 124.97	0.75 to 0.97	0.84 ± 0.06
11	974.94 ± 204.47	748.30 ± 111.66	0.62 to 0.99	0.79 ± 0.11
12	1078.59 ± 253.03	810.49 ± 220.36	0.45 to 0.87	0.69 ± 0.15
13	1244.48 ± 211.53	1016.76 ± 288.83	0.38 to 0.91	0.79 ± 0.16
14	1272.36 ± 158.66	1127.59 ± 164.93	0.80 to 0.94	0.87 ± 0.04
15	1257.79 ± 162.63	1064.98 ± 156.77	0.80 to 0.89	0.84 ± 0.03
16	1124.22 ± 161.46	927.85 ± 131.39	0.78 to 0.92	0.84 ± 0.03
17	960.18 ± 156.02	757.55 ± 170.88	0.70 to 0.84	0.79 ± 0.05
18	773.60 ± 200.79	648.62 ± 163.58	0.74 to 1.00	0.84 ± 0.09
19	618.75 ± 156.45	503.35 ± 143.60	0.72 to 0.87	0.79 ± 0.05
20	484.84 ± 143.36	424.19 ± 106.35	0.70 to 0.92	0.77 ± 0.07
21	417.35 ± 136.37	404.36 ± 110.66	0.73 to 0.80	0.78 ± 0.03
22	309.82 ± 107.38	455.31†	0.77†	0.77†

* <LOQ, less than the limit of quantification (208 ppb)
† Quantifiable concentrations only present in one egg
‡ NC, not calculable because parent fluralaner was less than the limit of quantification.

The data from this study indicate that parent fluralaner is an appropriate marker residue for monitoring the human food safety of eggs obtained from laying hens treated with fluralaner and that an egg tolerance of 2500 ppb is protective of human health.

b. Comparative Metabolism Study

Title: A Pivotal Comparative Metabolism Study in the Beagle Dog Following Multiple Oral Administration of 2 mg/kg Body Weight of [^{14}C]-Fluralaner. (Study No. S13344-00-HIC-MET-CN)

Study Dates: April 03, 2014 to October 14, 2015

Study Location: Tranent, East Lothian, United Kingdom

Study Design:

Objective: The objective of this study was to confirm that the toxicological animal species used to establish the ADI (*i.e.*, dogs) was exposed to the major metabolites detected in edible chicken meat tissues and eggs (*i.e.*, autoexposure).

Study Animals: One male beagle dog (6 months of age; 11.13 kg) and one female beagle dog (6 months of age; 8.67 kg) were used in this study.

Experimental Design: The study was conducted in accordance with GLPs (21 CFR § 58).

Drug Administration: Dogs were dosed by oral gavage with a solution containing [^{14}C]-fluralaner at a dose of 2 mg fluralaner/kg bw/day for 7 consecutive days.

Measurements and Observations: Samples of urine and feces were collected daily. Blood was sampled immediately prior to euthanasia. On Study Day 7, the dogs were euthanized by an overdose of barbiturate. Muscle, liver, fat, and kidney samples were collected.

Samples were extracted and subjected to metabolite profiling by radio HPLC.

In addition, metabolites corresponding to M5 and M10 detected in chicken tissues and eggs were isolated in dog liver and kidney samples by liquid chromatography and structurally elucidated by high resolution mass spectrometry.

Results: The major metabolites detected in chicken tissues and eggs (M5, mono-hydroxylated fluralaner and M10, fluralaner carboxylic acid) were detected by high resolution mass spectrometry in liver and kidney samples collected from dogs administered fluralaner.

Conclusions: Dogs orally administered fluralaner are exposed to the fluralaner metabolites that humans can be exposed to through the consumption of meat and eggs produced by chickens treated with fluralaner. Therefore, the ADI determined from data generated in dogs is appropriate for assessing the human food safety of meat and eggs from chickens treated with fluralaner.

c. Studies to Establish the Withdrawal Period and Egg Discard Time

(1) Tissue Residue Depletion Study

Title: Residue Depletion Study for Fluralaner Residues in Broiler Chickens Following Administration of Exzolt™ in Drinking Water. (Study No. S19059-00)

Study Dates: October 21, 2020 to September 12, 2022

Study Locations:

In-life phase: Tulare, CA
Water analysis: Greenfield, IN
Tissue analysis: Rahway, NJ

Study Design:

Objective: The objective of this study was to determine parent fluralaner concentrations in chicken muscle, liver, skin/fat, and kidney tissues following administration of fluralaner at a nominal dose of

0.60 mg fluralaner/kg bw offered as medicated water for 6 hours ± 30 minutes on two occasions spaced seven days apart.

Study Animals: Eighty Cobb 500 broiler chickens (40 males and 40 females) were used in this study. Chickens were 19 days of age and weighed 652.81 ± 39.53 g at the start of the study.

Experimental Design: The study was conducted in accordance with GLPs (21 CFR § 58). Chickens were assigned to one of five treatment groups, one spare treatment group, or one control untreated group (Table IV.9.).

Table IV.9. Experimental Groups for Chickens Orally Administered Fluralaner at a Nominal Dose of 0.60 mg/kg bw Offered for 6 hours ± 30 minutes on Two Occasions Spaced Seven Days Apart

Group	Number of Chickens	Slaughter Time
1 (control)	5 males and 5 females	Study Day 5
2	5 males and 5 females	1 day after last dose
3	5 males and 5 females	2 days after last dose
4	5 males and 5 females	4 days after last dose
5	5 males and 5 females	5 days after last dose
6	5 males and 5 females	10 days after last dose
7 (spare chickens)	10 males and 10 females	As needed

Drug Administration: Chickens were offered medicated water containing fluralaner for 6 hours ± 30 minutes on two occasions spaced seven days apart. The concentration of fluralaner in the medicated water was 9.9 µg/mL and 15.3 µg/mL on the first and second dosing occasion, respectively. The nominal dose on each occasion was 0.60 mg fluralaner/kg bw.

Measurements and Observations: At the assigned withdrawal period (Table IV.9.), chickens were slaughtered by electrical stunning followed by exsanguination. Samples of muscle, liver, skin/fat, and kidney were collected from each chicken.

Concentrations of parent fluralaner were determined in individual samples by LC-MS/MS using a validated analytical procedure.

Statistical Methods: Regression analysis was performed on liver and muscle data separately. Then, the upper tolerance limit for the 99th percentile with 95% confidence (99/95 UTL) was calculated from each of the regression models generated for liver and muscle.

Results: Parent fluralaner was detected in the samples collected and depleted as the withdrawal period progressed (Table IV.10.). The muscle and liver 99/95 UTLs were less than the muscle and liver tolerances determined from Study S18221-00, respectively, at 7 and 11 days after the last dose, respectively.

Table IV.10. Mean (± standard deviation) Concentrations (ppb) of Parent Fluralaner in Tissues from Chickens Offered Medicated Water Containing Fluralaner for 6 hours ± 30 minutes at a Nominal Dose of 0.60 mg/kg bw on Two Occasions Spaced Seven Days Apart

Withdrawal Period (days)	Muscle	Liver	Skin/Fat	Kidney
1	193.6 ± 67.0	1331.5 ± 408.7	923.4 ± 245.6	739.9 ± 225.5
2	125.5 ± 23.0	849.0 ± 144.1	563.4 ± 114.3	472.6 ± 70.0
4	87.1 ± 30.3	568.2 ± 182.0	387.0 ± 132.5	348.4 ± 76.3
5	60.1 ± 23.4	407.2 ± 164.9	263.1 ± 133.7	293.8 ± 48.3‡
10	24.3 ± 11.2*	128.0 ± 73.6	153.0 ± 46.7†	<LOQ§

* Only eight samples were greater than the limit of quantification (10 ppb)
† Only three samples were greater than the limit of quantification (71.9 ppb)
‡ Only four samples were greater than the limit of quantification (217 ppb)
§ <LOQ, less than the limit of quantification (217 ppb)

Conclusions: The data support an 11-day withdrawal period for laying hens and replacement chickens offered medicated water containing fluralaner for 6 to 24 hours at a dose of 0.50 mg fluralaner/kg bw on two occasions spaced seven days apart.

(2) Egg Residue Depletion Study

Title: Residue Depletion Study for Fluralaner Residues in Laying Hens Following Administration of Exzolt™ in Drinking Water. (Study No. S19060-00)

Study Dates: August 10, 2020 to July 25, 2022

Study Locations:

In-life phase: Tulare, CA
Water analysis: Greenfield, IN
Tissue analysis: Rahway, NJ

Study Design:

Objective: The objective of this study was to determine parent fluralaner concentrations in chicken eggs following administration of fluralaner at a nominal dose of 0.60 mg fluralaner/kg bw offered as medicated water for 6 hours ± 30 minutes on two occasions spaced seven days apart.

Study Animals: Twenty-five Bovans Brown laying hens were used in this study. Hens were 6 months of age and weighed 1897.12 ± 73.58 g at the start of the study.

Experimental Design: The study was conducted in accordance with GLPs (21 CFR § 58).

Drug Administration: Hens were offered medicated water containing fluralaner for 6 hours ± 30 minutes on two occasions spaced seven days apart. The concentration of fluralaner in the medicated water was 12.3 µg/mL and 12.9 µg/mL on the first and second dosing occasion, respectively. The nominal dose on each occasion was 0.60 mg fluralaner/kg bw.

Measurements and Observations: Eggs were collected twice daily from Study Day 0 through Study Day 22.

Concentrations of parent fluralaner were determined in individual samples by LC-MS/MS using a validated analytical procedure.

Statistical Methods: For each day of egg collection, the 99/95 UTL was calculated.

Results: Fluralaner was not quantifiable in eggs until Study Day 3, after which fluralaner concentrations began to increase with time. Fluralaner concentrations first peaked at Study Day 6.27 ± 0.59 with a concentration of 428.47 ± 95.83 ppb. This was followed by a brief decline in fluralaner concentrations. Then, following the second dose, a second peak occurred at Study Day 12.63 ± 0.67 with a concentration of 615.47 ± 104.66 ppb. Fluralaner concentrations declined after the second peak (Table IV.11.). The 99/95 UTLs were less than the egg tolerance (2500 ppb) determined from Study S18222-00-HID-STA-PO at all time points.

Conclusions: The data support a 0-day egg discard time for laying hens offered medicated water containing fluralaner for 6 to 24 hours at a dose of 0.50 mg fluralaner/kg bw on two occasions spaced seven days apart.

Table IV.11. Mean (± standard deviation) Concentrations of Parent Fluralaner in Eggs from Laying Hens Offered Medicated Water Containing Fluralaner for 6 hours ± 30 minutes at a Nominal Dose of 0.60 mg/kg bw on Two Occasions Spaced Seven Days Apart

Study Day	Number of Eggs Containing Quantifiable Concentrations	Fluralaner (Mean ± Standard Deviation; ppb)
0	0	<LOQ*
1	0	<LOQ
2	0	<LOQ
3	0	252.2 ± 39.3
4	13	309.6 ± 72.8
5	14	391.1 ± 84.6
6	14	418.5 ± 98.9
7	16	410.2 ± 85.9
8	15	348.0 ± 77.4
9	15	394.6 ± 80.8

Study Day	Number of Eggs Containing Quantifiable Concentrations	Fluralaner (Mean ± Standard Deviation; ppb)
10	14	473.9 ± 101.1
11	16	532.7 ± 105.6
12	15	598.3 ± 105.1
13	15	607.5 ± 107.3
14	14	562.9 ± 95.5
15	16	486.0 ± 76.1
16	15	385.3 ± 74.6
17	14	318.0 ± 66.2
18	12	277.1 ± 62.7
19	4	262.5 ± 55.2
20	4	248.3 ± 23.0
21	1	229[†]
22	0	<LOQ

* <LOQ, less than the limit of quantification (208 ppb)
† Only one egg sample contained quantifiable residues.

2. Target Tissue and Marker Residue

   The data from Study S18221-00 indicate that, for edible chicken meat tissues, the target tissue is chicken liver and that the marker residue is parent fluralaner. Study S18221-00 also indicates that parent fluralaner is an appropriate marker residue in chicken muscle.

   The data from Study S18222-00-HID-STA-PO indicate that parent fluralaner is an appropriate marker residue in chicken eggs.

3. Tolerances

   The data from Study S18221-00 support assigning a 320-ppb tolerance in chicken liver and a 110-ppb tolerance in chicken muscle.

   The data from Study S18222-00-HID-STA-PO support assigning a 2500-ppb tolerance in chicken eggs.

4. Withdrawal Period and Egg Discard Time

   The data from Study S19059-00 support an 11-day withdrawal period for laying hens and replacement chickens offered medicated water containing fluralaner for 6 to 24 hours at a dose of 0.50 mg fluralaner/kg bw on two occasions spaced seven days apart.

   The data from Study S19060-00 support assigning a 0-day egg discard time to laying hens offered medicated water containing fluralaner for 6 to 24 hours at a dose of 0.50 mg fluralaner/kg bw on two occasions spaced seven days apart.

F. Analytical Method for Residues

1. Description of Analytical Method

a. Determinative Procedure

One gram of homogenized chicken liver, egg, or muscle is spiked with deuterated fluralaner internal standard and extracted once with 2.9 mL of extraction solvent (acetonitrile/water, 80/20, v/v). Extraction supernatant (600 µL) is combined with 600 µL of water, followed by sample purification using solid-phase extraction. The resulting solution is quantitatively analyzed by gradient reversed phase LC-MS/MS using positive ion multiple reaction monitoring (MRM). The following ion transitions are monitored for quantitation:

Fluralaner: m/z 556 → m/z 400
Fluralaner-d4: m/z 560 → m/z 400

b. Confirmatory Procedure

Sample extraction for the confirmatory procedure is identical to the one for the determinative procedure. The resulting solution is quantitatively analyzed by gradient reversed phase LC-MS/MS using positive ion MRM. The following ion transitions are monitored for confirmation:

m/z 556 → m/z 457
m/z 556 → m/z 400
m/z 556 → m/z 160

2. Availability of the Method

The validated analytical method for analysis of residues of fluralaner is on file at the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855. To obtain a copy of the analytical method, please submit a Freedom of Information request to: https://www.accessdata.fda.gov/scripts/foi/FOIRequest/requestinfo.cfm.

V. USER SAFETY

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

Not for use in humans. Keep this and all drugs out of the reach of children.

Protective gloves should be used. Care should be taken when handling the product to avoid skin and eye exposure, exposure of mucous membranes, and accidental ingestion. Accidental exposure may cause skin and eye irritation. In case of eye contact, immediately rinse thoroughly with water. If wearing contact lenses, immediately rinse the eyes first, then remove contact lenses and continue to rinse the eyes thoroughly. Seek medical advice if symptoms occur. Wash hands and contacted skin with soap and water after use of the product. Remove contaminated clothes and launder with detergent.

Accidental ingestion may cause gastrointestinal disturbances and hypersensitivity reactions in humans.

To obtain a copy of the Safety Data Sheet (SDS) or for technical assistance, call Merck Animal Health at 1-800-211-3573.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that Exzolt™, when used according to the label, is safe and effective for the conditions of use in the General Information Section above. Additionally, data demonstrate that residues in food products derived from species treated with Exzolt™ will not represent a public health concern when the product is used according to the label.

A. Marketing Status

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to monitor the safe and effective use of this product.

B. Exclusivity

Exzolt™, as approved in our approval letter qualifies for THREE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of Exzolt™.

C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.