Date of Approval: October 2, 2015

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-449

SAFE-GUARD AquaSol

Fenbendazole oral suspension

Broiler chickens, replacement chickens intended to become breeding chickens, and breeding chickens

For the treatment and control of adult *Ascaridia galli* in broiler chickens and replacement chickens intended to become breeding chickens and for the treatment and control of adult *A. galli* and *Heterakis gallinarum* in breeding chickens.

Sponsored by:

Intervet, Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-449

B. Sponsor

Intervet, Inc.
2 Giralda Farms
Madison, NJ 07940

Drug Labeler Code: 000061

C. Proprietary Name

SAFE-GUARD AquaSol

D. Established Name

Fenbendazole oral suspension

E. Pharmacological Category

Anthelmintic

F. Dosage Form

Suspension

G. Amount of Active Ingredient

200 mg/mL

H. How Supplied

1 L and 3.875 L (1 gallon) high density polyethylene plastic containers

I. Dispensing Status

OTC

J. Dosage Regimen

1.0 mg/kg BW (0.454 mg/lb)/day for 5 consecutive days

K. Route of Administration

Oral in drinking water

L. Species/Class

Broiler chickens, replacement chickens intended to become breeding chickens, and breeding chickens

M. Indication

For the treatment and control of adult *Ascaridia galli* in broiler chickens and replacement chickens intended to become breeding chickens and for the treatment and control of adult *A. galli* and *Heterakis gallinarum* in breeding chickens.

II. EFFECTIVENESS

A. Dosage Characterization

Three dose determination studies were conducted to determine the dose and duration of SAFE-GUARD AquaSol (fenbendazole oral suspension) for the treatment and control of adult *Ascaridia galli* and *Heterakis gallinarum* in chickens. In these studies, effectiveness of different doses and durations of SAFE-GUARD AquaSol in medicated drinking water was evaluated in layer breed chickens. The dosage regimens evaluated include:

1. 5, 10, and 15 mg fenbendazole/kg body weight (BW)/day for 1 day,
2. 10 mg fenbendazole/kg BW for 3 days and 15 mg fenbendazole/kg BW for 5 days,
3. 1, 2, and 3 mg fenbendazole/kg BW/day for 5 consecutive days, and
4. 1 mg fenbendazole/kg BW/day for 7 consecutive days.

The results of these studies showed that 1 mg fenbendazole/kg BW/day for 5 consecutive days was the minimum effective dose and duration combination against adult *A. galli* and *H. gallinarum* in chickens.

B. Substantial Evidence

Six dose confirmation studies, and five field effectiveness studies were conducted to evaluate the effectiveness of SAFE-GUARD AquaSol against adult *A. galli* in broiler chickens and replacement chickens intended for breeding chickens and against adult *A. galli* and *H. gallinarum* in breeding chickens. A combination of studies in different classes of chickens, including laying hens (i.e., a class that is physiologically equivalent to layer type breeding hens), was used in the determination of effectiveness against the three classes of chickens listed above. In all studies, the fenbendazole treatment was administered orally in drinking water. Fenbendazole concentration in drinking water was calculated based on average daily water consumption and bird weights (average or total body weight of all birds in a group). Medicated drinking water was prepared fresh each day and fenbendazole concentration was analyzed in samples collected from each batch of medicated water using a validated method. Nematodes recovered from the ceca and intestines of birds at necropsy were identified and counted. All studies were conducted using naturally infected chickens in accordance with Good Clinical Practices as outlined in the VICH GL 9 Final Guidance (May 9, 2001). The comparisons were tested using a two-sided 5% significance level. The fixed model analysis was used to analyze log-counts, with treatment as a fixed effect. Percent efficacy was determined by comparing the geometric mean worm counts of the treated group (T) with those of the control group (C) for each parasite present in adequate numbers in at least six control animals using the formula: % efficacy = [(C-T/C) X 100].

To establish effectiveness for an indication, a minimum of two studies was required that meet the following criteria: an adequate level of infection in at least 6 control animals, the treatment effect was significant at alpha = 0.05, and 90% or greater efficacy using geometric means for each species of parasite. If there were more than two studies with an adequate level of infection, then the geometric means of the percent efficacy against a species of parasite from each study were added together and divided by the number of studies with that species of parasite. If this average was greater than or equal to 90%, then the drug was determined to be effective for that indication.

The results of the pivotal dose confirmation and field effectiveness studies demonstrate effectiveness of SAFE-GUARD AquaSol when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days against adult *A. galli* in broiler chickens and replacement chickens intended to become breeding chickens and against adult *A. galli* and *H. gallinarum* in breeding chickens. While effectiveness was also demonstrated for laying hens, this class is not included in this original approval because Human Food Safety technical section is not complete for use in laying hens.

The individual effectiveness studies are summarized below.

B.1 Study Number V-0079-1987

1. Type of study: Dose confirmation study in laying hens with naturally acquired nematode infections.

2. Investigator: Jennifer Ketzis, PhD, Charles River Laboratories Preclinical Services Ireland Ltd., Ballina, Ireland

3. Study Design:

    a. Objective: To evaluate the effective dose of SAFE-GUARD AquaSol against adult *A. galli, H. gallinarum,* and *Capillaria* spp.

    b. Study animals and experimental design: One hundred five Rhode Island Red laying hens (approximately 2 years old) were randomly assigned to five treatment groups. Each treatment group included 1 pen of 21 birds. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 20 birds during treatment. The birds were obtained from a commercial supplier in Ireland. All 20 birds in each treatment group were housed in one pen.

    c. Infection: Animals naturally infected with *A. galli, H. gallinarum,* and *Capillaria* spp. were obtained from a source farm.

    d. Dosage: There were three dosage regimens evaluated, but only the dosage regimen of 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days is reported.

    e. Control: Non-medicated drinking water

f. Test duration: All birds were necropsied 7 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli* and *H. gallinarum*. *Capillaria* spp. was not evaluated for this approval. The percent efficacies are summarized in the tables below.

Table B.1.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	20	17	3.38	-
1 mg/kg BW/day for 5 days	20	2	0.07	97.9

Table B.1.2: *H. gallinarum* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	20	18	31.3	-
1 mg/kg BW/day for 5 days	20	1	0.06	99.8

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* and *H. gallinarum* in laying hens.

B.2 Study Number V-0079-3621

1. Type of study: Dose confirmation study in laying hens with naturally acquired nematode infections.

2. Investigator: Jennifer Ketzis, PhD, Charles River Laboratories Preclinical Services Ireland Ltd., Ballina, Ireland

3. Study Design:

   a. Objective: To evaluate the effective dose of SAFE-GUARD AquaSol against adult *A. galli, H. gallinarum,* and *Capillaria* spp.

   b. Study animals and experimental design: One hundred five Rhode Island Red laying hens (approximately 2 years old) were randomly assigned to five treatment groups. Each treatment group included 1 pen of 21 birds. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 20 birds during treatment. The birds were obtained from a commercial supplier in Ireland. All 20 birds in each treatment group were housed in one pen.

   c. Infection: Animals naturally infected with *A. galli, H. gallinarum,* and *Capillaria* spp. were obtained from a source farm.

d. Dosage: There were three dosage regimens evaluated, but only the dosage regimen of 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days is reported.

e. Control: Non-medicated drinking water

f. Test duration: All birds were necropsied 7 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli* and *H. gallinarum*. *Capillaria* spp. was not evaluated for this approval. The percent efficacies are summarized in the tables below.

Table B.2.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	19	15	5.87	-
1 mg/kg BW/day for 5 days	20	3	0.16	97.3

Table B.2.2: *H. gallinarum* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	19	19	111.8	-
1 mg/kg BW/day for 5 days	20	13	3.51	96.9

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* and *H. gallinarum* in laying hens.

B.3 Study Number V-0079-3637

1. Type of study: Dose confirmation study in laying hens with naturally acquired nematode infections.

2. Investigator: Jennifer Ketzis, PhD, Charles River Laboratories Preclinical Services Ireland Ltd., Ballina, Ireland

3. Study Design:

a. Objective: To evaluate the effective dose of SAFE-GUARD AquaSol against adult *A. galli, H. gallinarum,* and *Capillaria* spp.

b. Study animals and experimental design: One hundred five Rhode Island Red laying hens (approximately 2 years old) were randomly assigned to five treatment groups. Each treatment group included 1 pen of 21 birds.

One sentinel bird was necropsied from each pen prior to treatment and each pen contained 20 birds during treatment. The birds were obtained from a commercial supplier in Ireland. All 20 birds in each treatment group were housed in one pen.

c. Infection: Animals naturally infected with *A. galli, H. gallinarum,* and *Capillaria* spp. were obtained from a source farm.

d. Dosage: There were three dosage regimens evaluated, but only the dosage regimen of 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days is reported.

e. Control: Non-medicated drinking water

f. Test duration: All birds were necropsied 7 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli* and *H. gallinarum. Capillaria* spp. was not evaluated for this approval. The percent efficacies are summarized in the tables below.

Table B.3.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	19	17	7.39	-
1 mg/kg BW/day for 5 days	20	8	0.45	93.9

Table B.3.2: *H. gallinarum* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	19	19	54.7	-
1 mg/kg BW/day for 5 days	20	10	1.47	97.3

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* and *H. gallinarum* in laying hens.

B.4 Study Number C09-093-01

1. Type of study: Dose confirmation study in laying hens with naturally acquired nematode infections.

2. Investigator: Tom Yazwinski, PhD, Department of Animal Sciences, University of Arkansas, Fayetteville, AR

3. Study Design:

   a. Objective: To evaluate the effective dose of SAFE-GUARD AquaSol against adult *A. galli, H. gallinarum,* and *Capillaria obsignata.*

   b. Study animals and experimental design: Two hundred sixty-four Rhode Island Red laying hens (approximately 1 year old) were randomly assigned to three treatment groups. Each treatment group included 8 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Prairie Grove, AR.

   c. Infection: Animals naturally infected with *A. galli* and *H. gallinarum* were obtained from a source farm. The birds were placed on the litter obtained from a broiler breeder flock naturally infected with *C. obsignata.*

   d. Dosage: There were two dosage regimens evaluated, but only the dosage regimen of 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days is reported.

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied 7 to 8 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli* and *H. gallinarum*. *Capillaria obsignata* was not evaluated for this approval. The percent efficacies are summarized in the tables below.

   Table B.4.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	78	29	0.69	-
1 mg/kg BW/day for 5 days	78	1	0.01	98.7

   Table B.4.2: *H. gallinarum* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	78	73	28.4	-
1 mg/kg BW/day for 5 days	78	14	0.22	99.2

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* and *H. gallinarum* in laying hens.

B.5 Study Number S10037-02

1. Type of study: Dose confirmation study in male and female broiler chickens with naturally acquired nematode infections.

2. Investigator: Terry N. TerHune, DVM, PhD, HMS Veterinary Development, Inc., Tulare, CA

3. Study Design:

   a. Objective: To confirm the effective dose of SAFE-GUARD AquaSol against adult *A. galli*.

   b. Study animals and experimental design: One hundred seventy-six Cobb broiler chickens (approximately 4 to 5 weeks old) were randomly assigned to two treatment groups. Each treatment group included 8 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Nashville, AR.

   c. Infection: Animals naturally infected with *A. galli* were obtained from a source farm.

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied 7 to 8 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli*. The percent efficacies are summarized in Table B.5.1.

   Table B.5.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	79	45	4.0	-
1 mg/kg BW/day for 5 days	79	1	0.02	99.4

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* in broiler chickens.

B.6 Study Number S10037-03

1. Type of study: Dose confirmation study in male and female broiler chickens with naturally acquired nematode infections.

2. Investigator: Stephen Davis, DVM, Colorado Quality Research, Wellington, CO

3. Study Design:

   a. Objective: To confirm the effective dose of SAFE-GUARD AquaSol against adult *A. galli*.

   b. Study animals and experimental design: One hundred seventy-six Ross broiler chickens (approximately 4 to 5 weeks old) were randomly assigned to two treatment groups. Each treatment group included 8 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Batesville, AR.

   c. Infection: Animals naturally infected with *A. galli* were obtained from a source farm.

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied 7 to 8 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli*. The percent efficacies are summarized in Table B.6.1.

   Table B.6.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds	Positive birds	Worm count geometric mean	% efficacy
Control	76	67	5.3	-
1 mg/kg BW/day for 5 days	79	0	0	100

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* in broiler chickens.

B.7 Study Number S10103-00

1. Type of study: Field effectiveness study in male and female replacement chickens with naturally acquired nematode infections.

2. Investigator: Paolo Fiorini, DVM, Salvignano Sul Rubicone FC, Italy

3. Study Design:

   a. Objective: To confirm the effectiveness of SAFE-GUARD AquaSol against adult *A. galli* in replacement chickens under field conditions.

   b. Study animals and experimental design: The study was conducted in a flock of 13,244 Hy-Line layer breed replacement chickens (13 weeks old). Prior to treatment, 15 birds were randomly selected from the flock house for necropsy and were used as the control group. The remaining birds in the flock were treated with the test article for 5 consecutive days. Seven days after the last treatment, 15 birds were randomly selected from the flock for necropsy and were used as the treated group. The chickens were housed in floor pens with straw as the litter material.

   c. Infection: The flock was naturally infected with *A. galli*.

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: A control article was not used in the study.

   f. Test duration: The 15 treated birds were necropsied 7 days after the last day of treatment.

4. Results: Eleven of the total 15 control birds necropsied prior to treatment were infected with at least one adult *A. galli*. The percent efficacies are summarized in Table B.7.1.

   Table B.7.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	15	11	1.15	-
1 mg/kg BW/day for 5 days	15	1	0.11	90.2

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* in replacement chickens.

B.8 Study Number S11328-00

1. Type of study: Field effectiveness study in male and female broiler chickens with naturally acquired nematode infections.

2. Investigator: Michael Sims, BS, Virginia Diversified Research and Consulting, LLC, Harrisonburg, VA

3. Study Design:

   a. Objective: To confirm the effectiveness of SAFE-GUARD AquaSol against adult *A. galli* in broiler chickens under field conditions.

   b. Study animals and experimental design: Five hundred fifty Ross broiler chickens (approximately 4 to 5 weeks old) were randomly assigned to two treatment groups. Each treatment group included 25 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Baker Hill, AL. The birds were housed in floor pens with wood shavings for litter.

   c. Infection: Animals naturally infected with *A. galli* were obtained from a source farm.

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied seven days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli*. The percent efficacies are summarized in Table B.8.1.

   Table B.8.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	100	52	2.3	-
1 mg/kg BW/day for 5 days	100	0	0	100

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* in broiler chickens.

B.9 Study Number S11334-00

1. Type of study: Field effectiveness study in male and female replacement chickens with naturally acquired nematode infections.

2. Investigator: Terry N. TerHune, DVM, PhD, HMS Veterinary Development, Inc., Tulare, CA

3. Study Design:

   a. Objective: To confirm the effectiveness of SAFE-GUARD AquaSol against adult *A. galli* and *H. gallinarum* in replacement chickens under field conditions.

   b. Study animals and experimental design: Five hundred fifty White leghorn replacement male and female chickens (approximately 14 weeks old) were randomly assigned to two treatment groups. Each treatment group included 25 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Rudd, IA.

   c. Infection: The flock was naturally infected with *A. galli* and *H. gallinarum.*

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied 7 days after the last day of treatment.

4. Results: The control birds necropsied prior to treatment were adequately infected with *A. galli* and *H. gallinarum*. The percent efficacies are summarized in the tables below.

Table B.9.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	100	30	0.3	-
1 mg/kg BW/day for 5 days	100	0	0	100

Table B.9.2: *H. gallinarum* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	100	89	5.5	-
1 mg/kg BW/day for 5 days	100	38	0.6	88.9

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* in replacement chickens.

B.10 Study Number S11335-00

1. Type of study: Field effectiveness study in female broiler breeder chickens with naturally acquired nematode infections.

2. Investigator: Steve Davis, DVM, Colorado Quality Research, Wellington, CO

3. Study Design:

   a. Objective: To confirm the effectiveness of SAFE-GUARD AquaSol against adult *A. galli* and *H. gallinarum* in broiler breeder chickens under field conditions.

   b. Study animals and experimental design: Five hundred fifty Cobb broiler breeder hens (approximately 63 weeks old) were randomly assigned to two treatment groups. Each treatment group included 25 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Checotah, OK.

   c. Infection: The flock was naturally infected with *A. galli* and *H. gallinarum.*

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied 7 days after the last day of treatment.

4. Results: The control birds necropsied prior to treatment were adequately infected with *A. galli* and *H. gallinarum*. The percent efficacies are summarized in the tables below.

   Table B.10.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	100	19	0.29	-
1 mg/kg BW/day for 5 days	100	1	0.01	97.6

   Table B.10.2: *H. gallinarum* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	100	91	19.3	-
1 mg/kg BW/day for 5 days	100	32	0.9	95.3

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* and *H. gallinarum* in broiler breeder chickens.

B.11 Study Number S12288-00

1. Type of study: Field effectiveness study in male and female broiler chickens with naturally acquired nematode infections.

2. Investigator: Kelly F. Lechtenberg, DVM, PhD, Midwest Veterinary Services, Inc., Oakland, NE

3. Study Design:

   a. Objective: To confirm the effectiveness of SAFE-GUARD AquaSol against adult *A. galli* in broiler chickens under field conditions.

   b. Study animals and experimental design: Five hundred fifty Cobb broiler chickens (approximately 4 to 5 weeks old) were randomly assigned to two treatment groups. Each treatment group included 25 pens containing 11 birds per pen. One sentinel bird was necropsied from each pen prior to treatment and each pen contained 10 birds during treatment. The birds were obtained from a commercial supplier in Eufaula, AL. The birds were housed in floor pens with wood shavings for litter.

   c. Infection: Animals naturally infected with *A. galli* were obtained from a source farm.

   d. Dosage: 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days

   e. Control: Non-medicated drinking water

   f. Test duration: All birds were necropsied 7 days after the last day of treatment.

4. Results: The control birds necropsied at the end of the study were adequately infected with *A. galli*. The percent efficacies are summarized in Table B.11.1.

   Table B.11.1: *A. galli* adult worm counts and efficacy of fenbendazole

Treatment groups	Total birds necropsied	Positive birds	Worm count geometric mean	% efficacy
Control	100	99	23.7	-
1 mg/kg BW/day for 5 days	100	0	0	100

5. Adverse reactions: No treatment related adverse reactions were observed during the study.

6. Conclusion: SAFE-GUARD AquaSol, when administered at 1 mg fenbendazole/kg BW/day in drinking water for 5 consecutive days, is effective against adult *A. galli* in broiler chickens.

III. TARGET ANIMAL SAFETY

The effects of SAFE-GUARD AquaSol administered orally via drinking water were evaluated in three target animal safety studies. Two margin of safety studies (one in broiler chickens and the other in laying hens, a class physiologically equivalent to layer type breeding hens) were conducted to evaluate the safety of SAFE-GUARD AquaSol administered orally via drinking water at 1, 3, and 5 mg fenbendazole/kg BW/day (1X, 3X, and 5X the labeled dose of 1 mg fenbendazole/kg BW/day) for 15 consecutive days (3X the labeled duration of 5 consecutive days). Reference intervals for clinical pathology parameters in broiler chickens and laying hens were generated using a separate reference interval study. A reproductive safety study was conducted to evaluate the safety of SAFE-GUARD AquaSol administered orally via drinking water to broiler breeder chickens at 3 mg fenbendazole/kg BW/day (3X the labeled dose of 1 mg fenbendazole/kg BW/day) for 21 consecutive days (4.2X the labeled duration of 5 consecutive days).

These studies demonstrated the target animal safety of SAFE-GUARD AquaSol in broiler chickens and replacement chickens intended to become breeding chickens, and breeding chickens when administered in drinking water at 1 mg fenbendazole/kg BW/day for 5 consecutive days. While target animal safety was also demonstrated for laying hens, this class is not included in this original approval because Human Food Safety technical section is not complete for use in laying hens.

The reference interval study, margin of safety studies, and reproductive safety study are summarized below.

A. Reference Interval Study

1. Title: "Clinical Pathology Reference Range in Broiler Chickens and Laying Hens." Study number S11357-00

2. Study Director: Sian Roberts, BSc, Charles River, Edinburgh, UK

3. Study Design:

   a. Objective: To provide hematology and clinical chemistry reference intervals for the interpretation of the clinical pathology results in margin of safety studies in broiler chickens (Study number S11298-00) and laying hens (Study number S11297-00).

   b. Study Animals: The study included two reference populations that accurately represented the populations for which the reference interval was used: broiler chickens and laying hens. All birds were housed and managed the same as the study animals in the margin of safety studies for broiler chickens and laying hens.

      Broiler chickens: Commercially bred, one day old Ross 308 strain chicks (109 male and 108 female) were obtained in three batches and evaluated

for study eligibility. Seventy healthy chicks (35 male and 35 female) from each batch were selected for enrollment into the study.

Sixty birds (30 male and 30 female) in each of the three batches, were randomly allocated to one of three blood sampling groups: hematology, clinical chemistry, and coagulation (coagulation samples were not analyzed). Remaining birds (5 male and 5 female per batch) were retained as spare birds to replace any birds that died following the start of blood sampling.

Laying hens: Ninety-nine Hy-line Brown laying hens were obtained in five batches and evaluated for study eligibility. Seventy-one healthy hens laying five good quality eggs per week were selected for enrollment into the study.

c. Measurements and Observations:

Trained personnel observed the general health of all birds and the litter moisture in each pen twice daily from arrival to the end of the study. Body weights (by pen for broiler chickens and individually for laying hens) were measured within 24 hours of arrival, at least every two weeks, and on each blood sampling day. For each batch of laying hens, eggs were collected twice daily and egg production recorded.

Blood collection for hematology and clinical chemistry: Blood was collected from each broiler chicken four times during the study. Table III.A.1 summarizes the blood collection time points for each batch of broiler chickens.

Table III.A.1: Blood collection from enrolled broiler chickens

Batch #	Total number of birds sampled	Hematology samples	Clinical chemistry samples	Blood sampling study days
1	20 M and 20 F	10 M and 10 F	10 M and 10 F	18, 26, 32, 38
2	21 M and 21 F	11 M and 10 F	10 M and 11 F	20, 27, 33, 37
3	20 M and 20 F	10 M and 10 F	10 M and 10 F	19, 28, 31, 36

Blood was collected from laying hens with a goal of complete hematology and clinical chemistry results from 40 hens. The blood collection from laying hens is summarized in Table III.A.2 below.

Table III.A.2: Blood collection from enrolled laying hens

Batch #	Total number of birds sampled	Hematology samples	Clinical chemistry samples	Blood glucose samples
1	15	15	15	-
2	12	12	12	-
3	10	10	10	10
4	16	6	-	15
5	18	3	3	15

The following hematology parameters were analyzed: total and differential white blood cell counts (heterophils, lymphocytes, monocytes, eosinophils, and basophils), red blood cell count, thrombocyte count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and hemoglobin.

The following clinical chemistry parameters were analyzed: albumin, aspartate aminotransferase, calcium, chloride, glutamate dehydrogenase, glucose, creatine phosphokinase, phosphate, potassium, sodium, total protein, uric acid, and globulin.

4. Clinical Pathology Analysis: The samples were collected, handled, and processed using standard procedures. The analytical methodology used for all hematology and clinical chemistry parameters were the same as those used in margin of safety studies in broiler chickens (Study number S11298-00) and laying hens (Study number S11297-00), and the analyses were performed using strict quality controls.

5. Statistical Analysis: Repeated measures linear models were used to incorporate the covariance among the four observations per broiler chicken. Reference intervals were determined based on 90% confidence limits on the overall means for the clinical chemistry and hematology parameters for broilers.

   Reference intervals for hematology, clinical chemistry, and glucose in laying hens were derived from the 90% confidence limits on the mean based on the sampling distribution for the number of hens available for each of the parameters.

6. Results: Reference intervals for each of the hematology and clinical chemistry parameters were established for Ross 308 broiler chickens based on a total of 240 values collected over four time-points from 61 birds. Reference intervals for Hy-Line Brown laying hens were established based on 46 hematology samples, 40 clinical chemistry samples, and 40 glucose samples from a total of 71 hens.

7. Conclusions: The health, age, genetics, production status, and environmental conditions of the reference interval population accurately represent the study populations from the margin of safety studies in broiler chickens and laying hens. The reference intervals generated in this study are acceptable for use in the interpretation of the clinical pathology results of the margin of safety studies in broiler chickens (Study number S11298-00) and laying hens (Study number S11297-00).

B. Margin of Safety Study in Broiler Chickens

1. Title: "Margin of Safety Study of 20% Fenbendazole Suspension in Drinking Water in Broiler Chickens." Study number S11298-00

2. Study Director: Ciara Vance BSc, Charles River, Edinburgh, UK

3. Study Design:

a. Objective: To demonstrate an adequate margin of safety for SAFE-GUARD AquaSol when administered orally via drinking water to broiler chickens at 1X, 3X, and 5X the labeled dose for 3X the labeled duration of treatment. The labeled dosage regimen is 1 mg fenbendazole/kg BW administered orally via drinking water for 5 consecutive days.

b. Study Animals: Commercially bred, one day old Ross 308 strain chicks (351 male and 351 female) were obtained for possible study inclusion. At arrival, birds were evaluated for study eligibility, and only healthy chicks were selected for enrollment into the study. The birds were approximately 23 days old when the dosing period started.

c. Treatment Groups: Four hundred eighty birds (240 male and 240 female) were selected for inclusion into the study. The birds were randomly allocated into pens of 10 birds each, with 12 pens in each of four treatment groups. Table III.B.1 summarizes the allocation of birds into the different treatment groups.

Table III.B.1: Treatment groups

Treatment groups	Number of pens	Number of birds/pen	Duration of treatment (days)
Control	12 (6 male, 6 female)	10	15
1 mg fenbendazole/kg BW/day	12 (6 male, 6 female)	10	15
3 mg fenbendazole/kg BW/day	12 (6 male, 6 female)	10	15
5 mg fenbendazole/kg BW/day	12 (6 male, 6 female)	10	15

d. Drug Administration: Fenbendazole was administered for 15 consecutive days in drinking water to target doses of 1, 3, and 5 mg/kg BW/day. The control article was non-medicated water and was administered continuously to the control group. Fenbendazole concentration in drinking water was calculated based on average daily water consumption and total body weight of all birds in each treatment group.

e. Measurements and Observations: Pens were observed twice daily to monitor the health of the birds. Assessments included litter moisture and excreta, body condition, eyes, respiration, nasal discharge, locomotion, skin and feathers, and behavioral attitude. Birds showing abnormal signs were examined by a veterinarian. Routine veterinary clinical observations were performed on Study Days -2, 2, 7, and 15 on all birds. Mortality rate was calculated, and gross necropsies were performed on all dead birds to determine the cause of death.

Feed consumption was recorded once daily during the duration of the study. Water consumption was measured for each pen from Study Day -7 until euthanasia on Day 16. The overall body weight of each pen

(combined body weight of all birds within a pen) was taken on the day of arrival and on Study Days -14, -7, -3, 6, 11, and 16.

At the start of the study, 24 birds from each treatment group (two birds per pen) were randomly selected for hematology evaluation, and 24 birds from each treatment group (two birds per pen) were randomly selected for clinical chemistry evaluation. The birds selected for hematology or clinical chemistry evaluations were subject to blood sampling on Study Days -3, 6, and 16. Gross necropsies were performed on 48 pre-selected birds from each treatment group (four birds from each pen). Histopathologic examinations were 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.

4. Statistical Analysis: Fenbendazole treated groups were compared to the control group. Statistical tests were two-sided and performed at a 0.10 significance level. No adjustment was made for multiple comparisons. The statistical unit was the pen.

   Body weights, feed and water consumption, clinical chemistry, and hematology variables were analyzed using repeated measures mixed models with the pre-treatment values as covariates and treatment, time, sex, and their interactions as fixed effects. Pairwise comparisons were performed between each of the treated groups and the control group within sex and day, within sex, within day, or overall, depending upon sequential evaluation of whether the three-way interaction, the treatment by sex interaction, the treatment by time interaction, or only the treatment main effect, respectively, was significant in the model. If none of the effects involving treatment group was significant, the analysis process was terminated without a statistical finding effect for that variable.

5. Results: No clinical signs of toxicity were observed. There were no treatment related effects on mortality and water consumption. Statistically significant differences in the control and treated groups were found for body weight and feed consumption; however the differences were not clinically relevant. Additionally, statistically significant differences in the control and treated groups were found for total WBC, heterophil, and basophil counts, and albumin values; however, the differences noted were not clinically relevant. Variations from the normal reference interval were noted for some hematology and clinical chemistry parameters; however, no fenbendazole related abnormalities were identified.

   Bird mortalities were attributable to common pathologies seen in broiler chickens and were observed in similar frequencies in the control and treated groups. No treatment related effects were observed during gross necropsy and histopathology evaluations.

   Fenbendazole concentration in each batch of medicated drinking water prepared daily was analyzed using a validated method. The mean daily dose

levels achieved for each fenbendazole-treated group were within 6% of the target dose levels of 1, 3 and 5 mg fenbendazole/kg BW/day.

6. Adverse Events: No test article related adverse events were observed during the study.

7. Conclusions: Based upon the results of this study, SAFE-GUARD AquaSol is safe in broiler chickens when administered orally in drinking water at 1 mg fenbendazole /kg BW/day for 5 consecutive days.

C. Margin of Safety Study in Laying Hens and Reproductive Safety Study in Breeding Chickens

a) Title: "Margin of Safety Study of 20% Fenbendazole Suspension in Drinking Water in Laying Hens." Study number S11297-00

1. Study Director: Ciara Vance BSc, Charles River, Edinburgh, UK

2. Study Design:

a. Objective: To demonstrate an adequate margin of safety for SAFE-GUARD AquaSol when administered orally via drinking water to laying hens at 1X, 3X, and 5X the labeled dose for 3X the labeled duration of treatment. The labeled dosage regimen is 1 mg fenbendazole/kg BW/day administered orally via drinking water for 5 consecutive days.

b. Study Animals: Hy-Line Brown laying hens (162 female) were obtained for possible study inclusion. At arrival, birds were evaluated for study eligibility, and only healthy hens laying five good quality eggs per week were selected for enrollment into the study. The birds were approximately 30 weeks old when the dosing period started.

c. Treatment Groups: One hundred forty-four hens were selected for inclusion into the study. The hens were individually housed in pens and randomly assigned to treatment groups. Each treatment group contained 36 individually housed hens. Table III.C.1 summarizes the allocation of hens into the different treatment groups.

Table III.C.1: Treatment groups

Treatment groups	Number of pens	Number of birds/pen	Duration of treatment (days)
Control	36	1	15
1 mg fenbendazole/kg BW/day	36	1	15
3 mg fenbendazole/kg BW/day	36	1	15
5 mg fenbendazole/kg BW/day	36	1	15

d. Drug Administration: Fenbendazole was administered for 15 consecutive days in drinking water to target doses of 1, 3, and 5

mg/kg BW/day. The control article was non-medicated water and was administered continuously to the control group. Fenbendazole concentration in drinking water was calculated based on average daily water consumption and total body weight of all birds in a group.

e. Measurements and Observations: Pens were observed twice daily to monitor the health of the birds. Assessments included litter moisture and excreta, body condition, eyes, respiration, nasal discharge, locomotion, skin and feathers, and behavioral attitude. Birds showing abnormal signs were examined by a veterinarian. Routine veterinary clinical observations were performed on Study Days -2, 2, 7, and 15 on all birds.

   Feed consumption was recorded for each bird once daily during the duration of the study. Water consumption was measured for each bird from Study Day -7 until the end of the in life phase of the study. The body weight of each bird was taken on the day the birds were assigned to the study, the day the birds were randomized, and on Study Days -7, -3, 6, 11, 16, and 23.

   Eggs were collected twice daily from each pen and visually inspected for soundness, egg height, egg width, egg height:width ratio, and presence of blood or meat spots. Egg shell thickness, egg strength, egg weight, albumen height, yolk color, and Haugh unit were measured. Egg abnormalities such as soft sells, misshapen eggs etc. were also recorded.

   At the start of the study, 12 birds from each treatment group were randomly selected for hematology evaluation, and 12 birds from each treatment group were randomly selected for clinical chemistry evaluation. The birds selected for hematology or clinical chemistry evaluations were subject to blood sampling on Study Days -3, 6, 11 and 16. Gross necropsies were performed on 12 pre-selected birds. Histopathologic examinations 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.

3. Statistical Analysis: Fenbendazole treated groups were compared to the control group. Statistical tests were two-sided and performed at a 0.10 significance level. No adjustment was made for multiple comparisons. The statistical unit was the bird.

   Body weights, feed and water consumption, egg production, egg weight, egg strength, eggshell thickness, Haugh unit, clinical chemistry, and hematology variables were analyzed using repeated measures mixed models with the pre-treatment values as covariates and treatment, time, and their interactions as fixed effects. Pairwise comparisons were performed between each of the treated groups and the control group within day or overall, depending upon sequential evaluation of whether

the treatment by day interaction or only the treatment main effect was significant in the model. If none of the effects involving treatment group were significant, the analysis process was terminated without a statistical finding of effect for that variable.

4. Results: No clinical signs of toxicity were observed. There were no treatment related effects on mortality, feed and water consumption, body weights, and egg production. A statistically significant difference in the control and treated groups was found for egg weight; however, the difference was not considered to be clinically relevant.

   There were no treatment related effects on the hematology and clinical chemistry parameters. Statistically significant differences in the control and treated groups were found for globulin and total protein values; however, the differences were not clinically relevant. Variations from the normal reference interval were noted for some hematology and clinical chemistry parameters; however, no fenbendazole related abnormalities were identified. No treatment related effects were observed during gross necropsy and histopathology evaluations.

   Fenbendazole concentration in each batch of medicated drinking water prepared daily was analyzed using a validated method. The mean daily dose levels achieved for each fenbendazole-treated group were within ±3% of the target dose level of 1, 3, and 5 mg fenbendazole/kg BW/day.

5. Adverse Events: No test article related adverse events were observed during the study.

b) Title: "Reproductive Safety Study of 20% Fenbendazole Suspension in Drinking Water Administration in Broiler Breeders." Study number S10050-00

1. Study Director: Terry N. TerHune DVM PhD, HMS Veterinary Development, Inc., Tulare, CA

2. Study Design:

   a. Objective: To demonstrate the reproductive safety of SAFE-GUARD AquaSol when administered orally via drinking water to broiler breeder chickens at 3X the labeled dose for 4X the labeled duration of treatment. The labeled dosage regimen is 1 mg fenbendazole/kg BW/day administered orally via drinking water for 5 consecutive days.

   b. Study Animals: Cobb strain broiler breeder chickens (30 male and 300 female) were acquired for the study. At arrival, animals were evaluated for study eligibility, and only healthy hens laying on average five good quality eggs per week and healthy males with consistent or increasing body weight were selected for enrollment into the study. The birds used were approximately 38 weeks old when the dosing period started.

   c. Treatment Groups: Two hundred twenty birds (20 male and 200 female) were selected for inclusion into the study. The birds were

randomly allocated into pens of 11 birds (1 male and 10 female in each pen), with 10 pens in each treatment group. Table III.D.1 summarizes the allocation of birds into the different treatment groups.

Table III.C.2: Treatment groups

Treatment groups	Number of pens	Number of birds/pen	Duration of treatment (days)
Control	10	11 (1 male and 10 female)	21
3 mg fenbendazole/kg BW/day	10	11 (1 male and 10 female)	21

d. Drug Administration: Fenbendazole was administered continuously for 21 days in drinking water to target a dose of 3 mg/kg BW/day. The control article was non-medicated water and was administered continuously to the control group. Fenbendazole concentration in drinking water was calculated based on average daily water consumption and total body weight of all birds in the treated group.

e. Measurements and Observations: Pens were observed twice daily to monitor the health of the birds including eyes, beaks, feathers, locomotion, and behavioral attitude. The temperature and humidity in the egg incubator and hatcher were recorded four times daily.

Feed consumption for each pen was recorded daily starting on arrival and continued through the 21-day treatment period. Water consumption for each pen was recorded daily starting two weeks before the treatment initiation and continued through the 21-day treatment period. The body weight of adult birds in each pen was recorded weekly throughout the study. The body weights of newly hatched chicks and 14 day old chicks were also recorded.

Daily egg production was recorded by pen, starting on Study Day 4 and continued through Study Day 140. Fertility % and hatchability % of the eggs laid during the dosing period were evaluated. Gross necropsies of unhatched eggs were performed to evaluate dead embryos and dead and culled hatchlings. Newly hatched chick viability evaluations included deformity, healed navel, dehydration, down color, ability to stand, and down dryness. Feather length of newly hatched chicks was also recorded. Thirty newly hatched chicks per treatment group were randomly selected for the evaluation of 14-day chick viability.

At the end of 21-day dosing period, 30 adult birds in each treatment group (1 male and 2 female per pen) were necropsied. The testes and female reproductive tracts were evaluated for gross pathology and weighed. The brain weight was also recorded for use in calculation of organ weights relative to brain weights. The duodenum and jejunum from male and female birds were examined for gross pathology. In addition, histopathologic evaluation of lesions collected during necropsy was performed.

3. Statistical Analysis: The fenbendazole treated group was compared to the control group. All statistical comparisons of the treatment main effect and two-way interaction of time with the treatment main effect were performed at a 0.10 significance level. The statistical unit was the pen for breeder chickens and newly hatched chicks.

   Pen weights, feed consumption, water consumption, egg production, and egg weights were analyzed by repeated measures mixed model analysis of covariance with pre-treatment observations used as covariates and treatment and time and their interaction as fixed effects. Pairwise comparison of the treated group to the control group was performed at each time, if the treatment by time interaction was significant. Egg fertility, hatchability, and chick viability parameters were binary outcomes analyzed with generalized linear mixed models with pre-treatment values for eggs from the same pen as covariates. Newly hatched chick weight was analyzed using an analysis of covariance with the newly hatched chick weights for chicks hatched previously from the same pen as the covariate, and 14-day chick viability was analyzed with a generalized linear mixed model without a covariate. Testes weights and female reproductive tract weights were analyzed by analysis of variance with treatment as the fixed effect.

4. Results: No clinical signs of toxicity were observed. There were no treatment related effects on mortality, feed consumption, body weights, egg production and weight, fertility, and hatchability. A statistically significant difference in the control and treated groups on Week 3 water consumption was noted; however, the difference was not considered to be clinically significant.

   There were no treatment related effects on the percentage of dead embryos, percentage of dead and culled hatchlings, percentage of newly hatched chick and 14-day old chick viabilities, and feather length of newly hatched chicks. No treatment related significant effects were observed during gross necropsy and histopathology evaluations.

   Fenbendazole concentration in each batch of medicated drinking water prepared daily was analyzed using a validated method. The mean daily dose levels achieved for the fenbendazole-treated group were within 1% of the target dose level of 3 mg fenbendazole/kg BW/day.

5. Adverse Events: No test article related adverse events were observed during the study.

c) Conclusions: Based upon the results of the margin of safety study in laying hens and the reproductive safety study in breeder chickens, SAFE-GUARD AquaSol is safe in breeding chickens when administered orally in drinking water at 1 mg fenbendazole/kg BW/day for 5 consecutive days.

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance

The Agency evaluated the need to address the impact of the use of fenbendazole on microbial food safety (antimicrobial resistance) among bacteria of public health concern in or on chickens. We considered that:

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

Therefore, the agency determined there was no need to develop or submit for review any microbial food safety (antimicrobial resistance, as described in Guidance for Industry #152, *Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern*) information regarding this use of fenbendazole in chickens.

B. Impact of Residues on Human Intestinal Flora

Residues and metabolites of fenbendazole are not known to have antimicrobial properties; additionally, residues and metabolites of fenbendazole have not been shown to impact bacterial populations. Therefore, at this time, the Agency does not think that residues and metabolites of fenbendazole in or on edible food products from treated animals will impact the intestinal flora of human consumers; thus, there was no need to submit additional information to address this human food safety endpoint.

C. Toxicology

Reassessment of the toxicological acceptable daily intake (ADI) was not needed for this approval. The FOI Summaries for the original approval of NADA 128-620 dated August 22, 1983, NADA 132-872 dated March 28, 1996, and NADA 131-675 dated February 10, 2000, contain relevant toxicology information.

D. Assignment of the Final ADI

The final ADI is the toxicology ADI of 40 µg/kg BW/day derived from a 6-month repeated dose oral toxicity study in dogs. The codified ADI is listed under 21 § CFR 556.275.

E. Safe Concentrations for Total Residues (edible tissues and injection sites, if applicable)

The calculation of the tissue safe concentrations is based on the *General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals* (FDA/CVM, revised July 2006) and reflects the partition (50% ADI for meat, 40% ADI for milk, and 10% ADI for eggs) requested by the drug sponsor.

The safe concentrations (ppm) of total fenbendazole residues in edible tissues are revised in this approval, in order to bring the total allowed fenbendazole residues in various edible tissues equal to one ADI (*i.e.*, 100% of the ADI). The safe concentration of total fenbendazole residues in each edible tissue of poultry is calculated using the following formula:

$$\text{Safe Concentration} = \frac{\text{percent partition x ADI (µg/kg bw/day) x 60 kg}}{\text{Consumption Value}}$$

The food consumption value is 300 g for muscle, 100 g for liver, 50 g for skin with adhering fat, and 100 g for eggs. The previous and the revised safe concentrations for total residues of fenbendazole in edible tissues and eggs of chickens are listed below:

Table IV.E.1. Safe concentrations for fenbendazole

Edible tissue	Previous safe concentration (ppm)	Revised safe concentration (ppm)
Muscle	8	4
Liver	24	12
Skin with adhering fat	48	24
Eggs	Not applicable	2.4

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Total Residue and Metabolism Studies

   The study described below was conducted to determine the levels of total residues in tissues of chickens following a six-day treatment with ^{14}C-fenbendazole administered orally. The dosing rate in the study was 5.0 mg/kg BW/day, which is five times the intended dose of 1.0 mg/kg BW/day.

   Study Number: 8084-01-06-94

   Study Director: Dr. Frederic W. Thalacker
   Corning Hazleton, Inc.
   Madison, Wisconsin

   Study Monitor: Dr. Robert J. Grant
   Hoeschst Roussel Vet
   Somerville, NJ

   A ^{14}C-fenbendazole metabolism and residue study was conducted using Peterson Arbor Acre Cross chickens that were approximately five weeks of age when fenbendazole treatment was initiated. Eighteen male and 18 female chickens were treated orally via gavage with 5.0 mg ^{14}C-fenbendazole/kg BW/day for six days. The radiolabeled fenbendazole was administered in two daily doses by gavage for six consecutive days at a concentration equivalent to 5.0 mg/kg BW per day. Six control chickens

(3 males, 3 females) were dosed with 0.05% Tween 80 (w/w) in deionized water.

Control chickens were slaughtered after administration of the last dose. Treated chickens were slaughtered 6, 12, 24, 48, 72, and 96 hours (six chickens per timepoint) after administration of the last dose. Excreta and cage wipes were collected at 24 hour intervals (or at 12 hours after the final dose and/or at termination if less than 24 hours from the last collection) from the day prior to dosing (excreta only) until termination. At termination, whole blood, liver, breast muscle, skin with adhering fat (skin/fat), abdominal fat, and the gastrointestinal tract and contents were collected. Serum was prepared by centrifugation of the clotted whole blood.

Concentrations of radioactivity and fenbendazole and the radiochemical purity of ^{14}C-fenbendazole in the dosing solutions were verified before and after the dosing period. All samples were analyzed for total radioactive residues (TRR). Cage and paper wipes, serum, abdominal fat, and skin/fat were analyzed for TRR directly by liquid scintillation counting (LSC). All other sample matrices were analyzed for TRR by combustion and LSC. Excreta and edible tissue samples were analyzed for total extractable and nonextractable residues by exhaustive solvent extraction. Metabolites were characterized in the extracts by HPLC and in-line LSC.

Results:

The concentrations of radioactivity in treated chickens reached a maximum at 6 hours post-dose. The highest concentrations of radioactivity in the examined tissues (Table IV.F.1) were in the liver (5.06 ppm), abdominal fat (1.48 ppm), skin with adhering fat (1.33 ppm), and serum (1.06 ppm). The breast muscle (1.01 ppm) also had detectable concentrations of radioactivity. No sex related differences were noted in the disposition of ^{14}C-fenbendazole.

The concentrations of radioactivity declined in tissues with time through the final 96 hour post-dose collection. Radioactivity was detected in all matrices, except breast muscle at 72 and 96 hours post-dose. Mean concentrations in the edible tissues are listed below in Table IV.F.1.

Table IV.F.1. Mean concentrations (ppm equivalents) of total radioactive residues (TRR) in tissues collected from chickens treated with ^{14}C-fenbendazole/kg BW/day for six days

Withdrawal period (hours)	Liver	Muscle	Abdominal fat	Skin/Fat
6	5.06	1.01	1.48	1.33
12	4.7	0.81	0.99	1.00
24	3.36	0.33	0.49	0.51
48	1.73	0.1	0.13	0.16
72	1.1	< 0.034	0.05	0.09
96	0.66	< 0.034	0.02	0.05

The majority of the administered radioactivity was eliminated in the excreta by 24 hours post-dose. The radioactivity recovered in the excreta was 97.6% of the total administered radioactivity for the males and 102% for the females. All of the examined tissues had less than 0.01% of the total administered radioactivity at 96 hours post-dose, with the exception of liver (0.06% in males and 0.08% in females).

Fenbendazole sulfone was the predominant metabolite present in all liver samples analyzed (Table IV.F.2). Oxfendazole was present in most of the early samples and a small quantity of unchanged fenbendazole was present in the 6-hr and 12-hr withdrawal samples. Small concentrations of p-hydroxyfenbendazole as peaks possibly mixed with other compounds also appeared to be present in some of the liver samples. Fenbendazole sulfone was the predominant residue present in all muscle samples analyzed. Smaller concentrations of oxfendazole were observed at the shorter withdrawal times, but no parent drug was detected in any of the samples. In skin with adhering fat, fenbendazole sulfone was the predominant metabolite in all samples analyzed, with a relatively small amount of parent fenbendazole in the 6hr samples. As with the skin/fat samples, fenbendazole sulfone was the predominant metabolite in all samples analyzed in abdominal fat, with some unchanged fenbendazole was present in the 6-hr fat samples.

Table IV.F.2. Metabolites of fenbendazole present (ppm equivalents) in the extracts of liver from chickens treated orally with ^{14}C-fenbendazole at 5 mg/kg BW for six consecutive days

Withdrawal period (hours)	Fenbendazole sulfone (ppm)	Oxfendazole (ppm)	*p*-Hydroxyfenbendazole (ppm)
6	2.41	0.61	Not detected
12	1.76	0.24	1.18
24	1.1	0.15	0.51
48	0.32	0.06	ND
72	0.09	0.03	0.03
96	0.01	Not detected	0.01

b. Comparative Metabolism Study

CVM did not require a comparative metabolism study for this approval. Results from the comparative metabolism studies for fenbendazole have been summarized in the original approval of NADA 128-620 dated August 22, 1983.

c. Study to Establish Withdrawal Period and/or Milk Discard Time

(1) Tissue Residue Depletion Study

CVM did not require a tissue residue depletion study for this approval. The data in study #8084-01-06-94 show that total residues of

fenbendazole in the edible tissues of chickens are well below the safe concentrations for residues in chickens.

2. Target Tissue and Marker Residue

The data in study #8084-01-06-94 summarized above show that liver is the edible tissue of chickens in which residues of fenbendazole are highest and persist longest. The study also demonstrated that fenbendazole sulfone is the residue present in the highest concentration and persists for the longest period of time. Thus, the target tissue is liver and the marker residue is fenbendazole sulfone.

3. Tolerances

Total residues of fenbendazole in liver were below the safe concentration of 12 ppm at the 6 hour withdrawal time point in the total residue and metabolism study #8084-01-06-94, and a marker to total residue ratio of 44% was observed. Thus, 5.2 ppm is assigned as the tolerance for fenbendazole sulfone in chicken liver.

4. Withdrawal Period

The results of study #8084-01-06-94 demonstrate that residues are below the safe concentration at 6 hours withdrawal (practical zero withdrawal). Therefore, the withdrawal period is zero.

G. Analytical Method for Residues

1. Description of Analytical Method

a. Determinative Procedure

After adding the deuterium-labeled internal standard (FBZ-sulfone-d3) to homogenized chicken liver, the sample is extracted twice with methanol by shaking on a vortex mixer. After centrifuge, the supernatant is diluted with methanol and analyzed using liquid chromatography with mass spectrometric detection (LC-MS/MS). Quantitation is based on the m/z 300 ion from FBZ-sulfone and FBZ-sulfone-d3.

b. Confirmatory Procedure

Sample extraction for the confirmatory procedure is identical to the one for the determinative procedure. Fenbendazole sulfone is detected using a tandem mass analyzer (MS/MS). Four fenbendazole sulfone-specific ions (m/z 332, m/z 330, m/z 159 and m/z 104) are monitored to obtain ion ratios, signal to noise ratios, and retention times that meet the required acceptability criteria.

2. Availability of the Method

The method is available from the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to SAFE-GUARD AquaSol:

"User Safety Warnings: Not for use in humans. Keep out of reach of children. Protective gloves should be used and care should be taken when handling the product to avoid skin and eye exposure and accidental ingestion. Accidental exposure may result in skin and eye irritation. Accidental ingestion may cause gastrointestinal disturbances and hypersensitivity reactions in humans. For customer service, adverse effects reporting, and/or copy of the SDS, call 1-800-211-3573. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS, or http://www.fda.gov/AnimalVeterinary."

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 SAFE-GUARD AquaSol, when used according to the label, is safe and effective for the treatment and control of adult *Ascaridia galli* in broiler chickens and replacement chickens intended to become breeding chickens and for the treatment and control of adult *A. galli* and *Heterakis gallinarum* in breeding chickens. Additionally, data demonstrate that residues in food products derived from species treated with SAFE-GUARD AquaSol will not represent a public health concern when the product is used according to the label.

A. Marketing Status

This product can be marketed over-the-counter (OTC) because the approved labeling contains adequate directions for use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

B. Exclusivity

SAFE-GUARD AquaSol, 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 FD&C Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of SAFE-GUARD AquaSol.

C. Patent Information:

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