

Internal Parasite Control in Commercial Layers

Safe-Guard® AquaSol: The First Anthelmintic Approved for Use in Pullets and Commercial Layers in Production

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Summary: Intestinal parasitic worms are a common problem in the poultry industry. These parasites have a significant impact, contributing to the transmission of disease, decreasing yield, and subsequently, increasing costs. This parasitism adds to the total price of production and results in significantly lower producer profits. Due to the systematic impact of intestinal worms, a water soluble fenbendazole (Safe-Guard® AquaSol) has been developed by Merck Animal Health and approved for use in poultry. This new product is a highly stable, farm-friendly, water-administered suspension. The stability of Safe-Guard® AquaSol does not require frequent agitation and provides a high level of efficacy as demonstrated in repeated animal tests. Gastro-intestinal nematodes including *Ascaridia galli* (L5 and adult stages) and *Heterakis gallinarum* (L5 and adult stages) are susceptible to treatment with Safe-Guard® AquaSol. A review of stability characteristics over time and efficacy in layers, broilers, breeders, layer pullets, and commercial layers will be discussed.

Keywords: *Ascaridia galli*, *Heterakis gallinarum*, nematode, *Histomonas*, blackhead, fenbendazole, AquaSol

Abbreviations: Fenbendazole (FBZ), United States (US)

Introduction: Like most vertebrates, chickens are vulnerable to parasites that can live on or in their bodies. Internal gastrointestinal helminths are a costly global problem for poultry production, capable of causing clinical disease, poor meat quality, and other negative economic impacts that severely erode the efficiency and profitability of producing meat and eggs. Parasitic nematodes populating the gastrointestinal system of production birds are by far the most significant group of helminths affecting poultry (2). The prevalence of gastrointestinal nematode infections within modern commercial poultry facilities are directly related to the environmental management, market age of the bird, production system, and, if utilized, effective parasiticides. These facilities in the United States (US) employ intervention strategies based predominantly on prophylactic treatment of long-lived bird populations and treat broilers on an as-needed basis on farms with a history of heavy nematodiasis (industry survey by author). Administration of these intervention strategies via drinking water is advantageous over in-feed medications due to the ability to treat poultry upon diagnosis of an active worm infestation. Prior to the recent introduction of AquaSol, piperazine was the only product labeled for use in poultry to treat nematodes. However, when used at the recommended dosage over 20 years ago, piperazine was shown to be 57.5% efficacious (1). This poor efficacy resulted in a significant percentage of the US poultry industry utilizing other anthelmintics off-label, requiring a veterinary prescription. These off-label products have been effective, but the physical stability and homogeneity of these treatments have been less than ideal, often resulting in sediment that obstructs bulk tanks, pipes, and watering system equipment resulting in potentially under dosed birds within the flock.

Fenbendazole (FBZ) is an excellent broad spectrum anthelmintic with proven efficacy in a variety of animal species (2). The new formulation of FBZ, Safe-Guard® AquaSol, recently released by Merck Animal Health in the United States is labeled for the treatment of gastrointestinal nematodes in chickens infected with the preadult L5 and adult stages of *Ascaridia galli* (roundworms) and the preadult L5 and adult stages of *Heterakis gallinarum* (cecal worms), two of the most important helminth species in poultry (2). A patented wet-milling process is utilized to reduce the active ingredient of Safe-Guard® AquaSol to a refined, homogenous, and submicron particle size allowing it to remain in suspension without agitation for up to 24 hours. Safe-Guard® AquaSol provides a safe and unique combination of dosing flexibility, efficacy, and convenience in a farm-friendly formulation developed for water-administration. Results are described herein.

Materials and Methods:

Efficacy: A negatively controlled, partially blinded, randomized, single-site dose-confirmation study was conducted to confirm the efficacy of three Safe-Guard® AquaSol dosage regimens administered in drinking water (1 and 2 mg/kg BW daily for 5 days, and 3.5 mg/kg BW daily for 3 days) for treating natural infections of

A. galli and *H. gallinarum* in chickens. Experimental birds consisted of 105 female chickens, approximately 2 years of age, weighing 1.3 to 2.5 kg.

On day 10, chickens were ranked by weight into 21 blocks of 5. Birds were then randomly divided into 5 treatment groups (20/group) with 5 sentinel birds randomly allocated for day 1 necropsy and worm counts. Treatments were administered via drinking water as follows: group 1: untreated control group (5-day dosing regimen), group 2: untreated control group (3-day dosing regimen), group 3: 1 mg FBZ/kg BW/d for 5 days (days 1-5), group 4: 2 mg FBZ/kg BW/d for 5 days (days 1-5), group 5: 3.5 mg FBZ/kg BW/d for 3 days (days 1-3). Fecal sampling was performed on days 7, 6, 4, and 3 for all groups, then on days 9 and 10 for groups 2 and 5, and on days 11 and 12 for groups 1, 3, and 4. Chickens were then euthanized and necropsied 7 days after last treatment administration (day 10 and day 12) for parasite collection. The nominal FBZ concentrations for the medicated water were calculated using the mean of individual BW and number of chickens per pen on day 3 and the average daily water consumption measured on day 6 to day 4. To ensure complete consumption of the medicated water, dosages were provided in approximately 50% of the normal daily amount of drinking water. Efficacy assessment was based on percent worm count reduction of the treated animals as compared to the negative controls at necropsy. Chickens used in this study were naturally infected with *A. galli* and *H. gallinarum*, as confirmed by the statistical and parasitological approaches (required at least 6 chickens in the 2 control groups for adequacy of infection). For statistical approaches, adequacy of infection was achieved for adult *A. galli* in study group 1 and *H. gallinarum* in study groups 1 and 2, but not for adult *A. galli* in study group 2. For parasitological approaches, adequacy of infection was achieved for pre-adult and adult stages of *A. galli* and *H. gallinarum*.

The study was not fully blinded due to the different number of days over which treatment was administered, but the level of blinding was deemed sufficient because 2 control groups were used (treated for 3 or 5 days). Three adverse events were reported in chickens treated with FBZ during the study but none were considered likely to be related to anthelmintic administration.

Safety: Test birds were randomly divided into 4 treatment groups and administered Safe-Guard® AquaSol in the drinking water at daily dose rates of 0, 1, 3, or 5 mg FBZ/kg BW, corresponding to dose rates of 0, 1×, 3×, or 5× the recommended dose rate, respectively. In addition, each dose rate was administered daily for 15 consecutive days, 3-times the recommended 5-day treatment duration. As a result, the total net doses of FBZ administered were 3×, 9×, or 15× the recommended total dose (Figure 1).

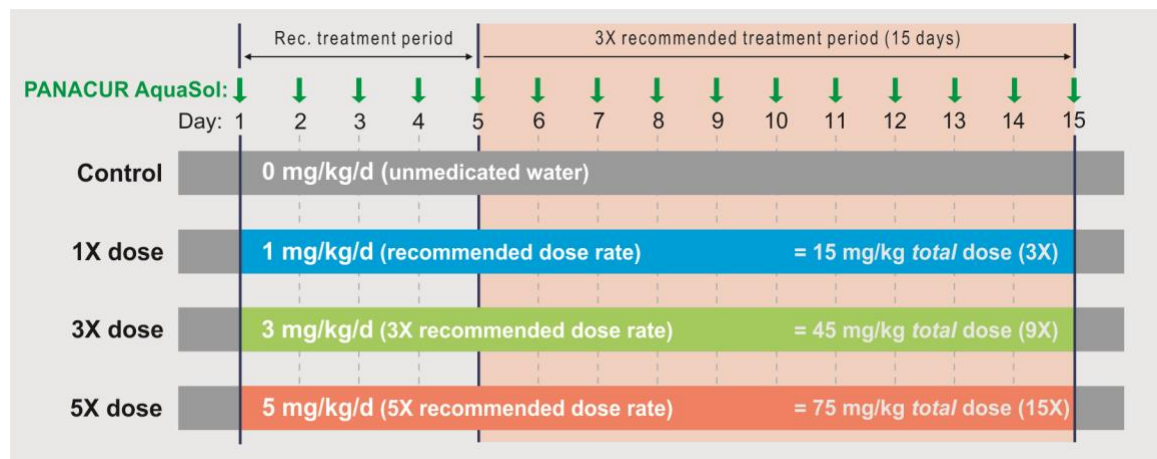


Figure 1: Experiment design of target animal safety studies.

This study involved 480 healthy broiler chickens (Ross 308, 240 males/240 females) housed in 48 single-sex pens holding 10 chickens/pen (each pen comprised an experimental unit). Six pens of each gender were randomly allocated to each of the 4 treatment groups (12 pens/treatment). Prior to treatment, 2 chickens/pen were randomly selected for hematology assessments, 2/pen for clinical chemistry samplings, and 4/pen for later necropsy. Treatment with Safe-Guard® AquaSol at the various dose rates commenced when chickens were 21 to 23 days of age, with daily treatment administered in drinking water for voluntary uptake over a full 24-hour period.

Clinical health was monitored throughout the study and feed and water intake were recorded. In addition, hematology and clinical chemistry parameters were measured pre-treatment (study day -3) and after commencement of treatment on study days 6 and 16. On study day 17, gross necropsies were performed on chickens identified before treatment and histopathology samples were collected. Data collections were performed by personnel blinded to treatment, with the exception of histopathology assessments.

Results:

Efficacy: All three Safe-Guard® AquaSol treatment groups provided significant ($P < 0.05$) worm count reductions compared to controls for both nematode species (Table 1). Efficacy $\geq 96.9\%$ was demonstrated in all treated groups. Results from groups treated with a 5-day regimen (1 or 2 mg/kg BW/day) were consistent with results from other dose-determination and dose-confirmation studies, while the 3-day dosage regimen (3.5 mg/kg BW/day) also demonstrated similarly high efficacy against the target parasites.

Table 1: Efficacy results of Safe-Guard® AquaSol against *A. galli* and *H. gallinarum*.

Study group (FBZ dose regimen) ^a	Pre-adult and adult worm count reduction (%)	
	<i>A. galli</i>	<i>H. gallinarum</i>
1,2 Untreated controls	---	---
3 1 mg/kg BW/d for 5 days*	97.3	96.9
4 2 mg/kg BW/d for 5 days	100.0	99.3
5 3.5 mg/kg BW/d for 3 days	100.0	99.4

^aWorm count reductions vs controls significant ($P < 0.05$) for both nematode species.

*Recommended Safe-Guard® AquaSol dose

Safety: Analysis of the medicated water samples confirmed that FBZ concentrations were 100% to 104% of the expected concentration for each treatment group. Evaluations of the massive quantity of collected data revealed no clinically significant differences for any safety or toxicological parameters between the control group and all medicated groups, including birds treated at the 5× daily dose rate. Thus, outcomes for feed consumption, body weights, hematology, clinical chemistry, organ weights, and histopathology were similar between all groups, and any occasional detected differences were not related to drug administration. Water intake was also similar between all treatment groups (Figure 2), indicating that normal palatability was maintained for drinking water medicated with Safe-Guard® AquaSol

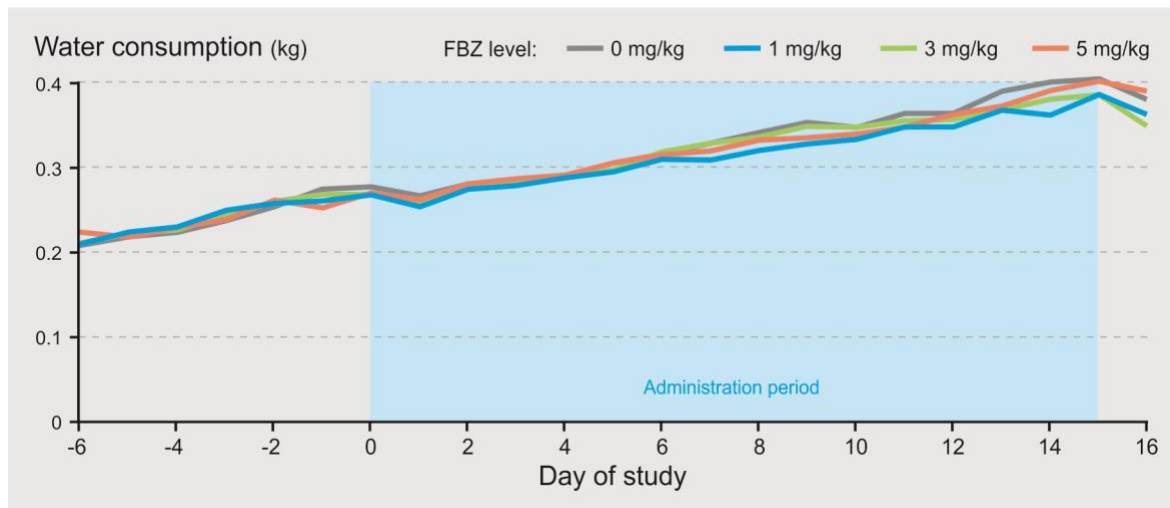


Figure 2: Similar intake of non-medicated and Safe-Guard® AquaSol -medicated drinking water by growing broiler chickens.

Discussion:

Multiple dose-confirmation studies have demonstrated potent and consistent efficacy of Safe-Guard® AquaSol treatment when provided in the drinking water of chickens at the most cost-effective daily dose and duration of 1 mg/kg BW for 5 consecutive days. In all trials, Safe-Guard® AquaSol treatment at the 1 mg/kg BW/day dosage provided significant ($P < 0.05$) worm count reductions compared to controls for both *A. galli* and/or *H. gallinarum*. Dosages greater than 1 mg/kg BW or for durations exceeding 5 days did not significantly improve efficacy. Thus, 1 mg FBZ/kg BW/day for 5 days represents the cost-effective recommended dosage for Safe-Guard® AquaSol, providing high and consistent efficacy against *A. galli* and *H. gallinarum* parasites. In addition, while *H. gallinarum* infections alone may not exert severe direct impacts on poultry health and

production, the parasites pose their greatest risk as carriers of *Histomonas meleagridis*, a protozoal agent that causes blackhead disease in chickens and turkeys. As an important transmission vector for *H. meleagridis* and (blackhead), cecal worms thus present a considerable threat to ongoing flock health and profitability that requires appropriate prevention and control measures, as such Safe-Guard® AquaSol stands as one of the few remaining tools to control this economically important disease.

The target animal safety study demonstrated that Safe-Guard® AquaSol is highly palatable and well tolerated in growing broiler chickens, even when grossly overdosed at 5-times the intended daily dose rate for 3-times the intended duration. Use of Safe-Guard® AquaSol at the recommended daily dose rate of 1 mg/kg BW for 5 days clearly offers a wide margin of safety for growing broiler chickens (and all classes of growing chickens) and does not impact rates of water consumption.

FBZ has been used for decades in multiple species and is known to be extremely safe for animals. Safe-Guard® AquaSol has been tested extensively in chickens, including during intense periods of growth and peak egg production. These and other studies have demonstrated the product is safe for use in all classes of chickens and that it has no adverse impact on fertility, hatchability, survival of progeny or water consumption. Safe-Guard® AquaSol clearly offers safe and consistent anthelmintic efficacy against two economically significant parasites affecting all classes.

References:

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