Aquatic Animal Drug Approval Partnership
4050 Bridger Canyon Road
Bozeman, MT 59715

10 mg/Kg of fish/day calculations

Date: 04/13/09

Form B. Determining the amount of Aquaflor® premix needed to treat fish in test tanks, based upon the amount of feed.

1) Amount of feed needed for treatment period
total weight of feed (lbs) _____ x conversion 453.6 = _____ g of feed needed for entire study

OR

total weight of feed (kg) _____ x 1000 = _____ g of feed needed for entire study

1. Determine % Aquaflor® premix by feeding rate (%BW)

Table 1. To determine the amount of Aquaflor® to add to either a 50 lb (22,680 g) or 20 kg (20,000 g) bag of fish feed, find the feeding rate (%BW) that fish will be fed, and the corresponding % Aquaflor®. Multiply the weight of feed/bag (g) by the % Aquaflor®, or move down to the third row to determine the amount of Aquaflor® to add to one 50 lb bag of feed, or the fourth row to determine the amount of Aquaflor® to add to one 20 kg bag of feed.

%BW	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	10.0
% Aquaflor®	0.400	0.200	0.133	0.100	0.080	0.066	0.057	0.050	0.044	0.040	0.020
Amount (g) / 50 lb bag	90.72	45.36	30.16	22.68	18.14	14.97	12.93	11.34	9.98	9.07	4.54
Amount (g) / 20 kg bag	80.00	40.00	26.60	20.00	16.00	13.20	11.40	10.00	8.80	8.00	4.00

Amount of Aquaflor® pre-mix in total amount of feed
(% Aquaflor premix _____ / 100) x total amount of feed (g) _____ = _____ g of Aquaflor®

4) Amount of oil to be used when mixing
total amount of feed (g) _____ x (0.5%) = _____ g of fish oil

Summary:

add _____ (g) Aquaflor® pre-mix (from Table 1 or bullet #3)

to _____ feed (#1)

mix with _____ (g) fish oil (#4)

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4050 Bridger Canyon Road
Bozeman, MT 59715

Date: 04/13/09

15 mg/Kg of fish/day calculations

Form B. Determining the amount of Aquaflor® premix needed to treat fish in test tanks, based upon the amount of feed.

1) Amount of feed needed for treatment period
total weight of feed (lbs) _____ x conversion 453.6 = _____ g of feed needed for entire study

OR

total weight of feed (kg) _____ x 1000 = _____ g of feed needed for entire study

1. Determine % Aquaflor® premix by feeding rate (%BW)

Table 1. To determine the amount of Aquaflor® to add to either a 50 lb (22,680 g) or 20 kg (20,000 g) bag of fish feed, find the feeding rate (%BW) that fish will be fed, and the corresponding % Aquaflor®. Multiply the weight of feed/bag (g) by the % Aquaflor®, or move down to the third row to determine the amount of Aquaflor® to add to one 50 lb bag of feed, or the fourth row to determine the amount of Aquaflor® to add to one 20 kg bag of feed.

%BW	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	10.0
% Aquaflor®	0.600	0.300	0.200	0.150	0.120	0.099	0.086	0.075	0.066	0.060	0.030
Amount (g) / 50 lb bag	136.08	68.04	45.24	34.02	27.21	22.46	19.40	17.01	14.97	13.61	6.81
Amount (g) / 20 kg bag	120.00	60.0	39.90	30.00	24.00	19.80	17.10	15.00	13.20	12.00	6.00

Amount of Aquaflor® pre-mix in total amount of feed
(% Aquaflor premix _____ / 100) x total amount of feed (g) _____ = _____ g of Aquaflor®

4) Amount of oil to be used when mixing
total amount of feed (g) _____ x (0.5%) = _____ g of fish oil

Summary:

add _____ (g) Aquaflor® pre-mix (from Table 1 or bullet #3)

to _____ feed (#1)

mix with _____ (g) fish oil (#4)

ORIGINAL

Miscellaneous Operating Procedures

I. **TITLE:** Procedure for Calculating and Top-coating AQUAFLOR® onto Feed

II. **DRUG INFORMATION:**

A. Product name: AQUAFLOR® Premix (50% florfenicol)

B. Manufacturer: Intervet Inc., a subsidiary of Merck & Co. Inc. Summit, NJ

III. **PRECAUTIONS:**

A. Before handling AQUAFLOR® Premix, be familiar with the MSDS.

B. Before top-coating AQUAFLOR® Premix onto feed, be trained to safely and properly use the Marion Laboratory Mixer according to SOP INST 126.

IV. **PROCEDURE:**

A. Calculations

Note: Unless otherwise stated, a % sign after a number always indicates the number preceding the % sign should be divided by 100 before calculating the equation.

1. Amount of feed needed for treatment
(# of fish to be treated) × (mean weight of fish, g) × (feeding rate, % body weight) × (treatment period, 10 days) = g of total feed

a. Example:

(5000 fish to be treated) × (4.26 g/fish) × (1.5% body weight) × (10 d) = 3,195 g of total feed (round up to 3,200 g = 3.2 kg)

b. For best results, make at least 5 kg of feed in the Marion Mixer (mixer holds up to 12 kg of feed).

2. Percentage of AQUAFLOR® Premix needed:
   (desired dosage*) / [(% active ingredient**) × (feeding rate, % body weight***)] = % AQUAFLOR® Premix

   * desired dosage = 10 – 15 mg florfenicol/kg fish biomass/d (*constant*)
   ** % active ingredient = 50% active (*constant*)
   *** The only number you need to plug into this equation is the % body weight (feeding rate) to feed your fish.

   a. Example:
      (10 mg/kg fish biomass/d) / [(50%) × (1.5% body weight)] = 0.133% AQUAFLOR® Premix in feed

      Note: For calculation in Example 2a, do not divide percentages by 100 before multiplying, just use the number (i.e., 1.5% = 1.5).

   b. Make at least 5 kg of feed in the Marion Laboratory Mixer (Section IV.A.1.a):
      (5000 g feed) × (0.133% AQUAFLOR® Premix) = 6.65 g AQUAFLOR® Premix to add to 5 kg of feed

3. Amount of fish oil needed:
   (total amount of fish feed, g) × (0.5%) = g of fish oil to spray onto feed

   a. Example:
      (5000 g feed) × (0.5%) = 25 g of fish oil

   b. Assume 1 mL of fish oil weighs 1 g; after weighing out appropriate amount, transfer to a spray bottle and warm in microwave for ~20 seconds so oil can be easily sprayed.

B. Top-coating AQUAFLOR® Premix onto feed

1. Pre-measure all ingredients (fish feed, AQUAFLOR® Premix, and fish oil).

2. Add appropriate amount of feed to mixer (between 5 and 12 kg).

3. Turn on mixer and slowly add AQUAFLOR® Premix near the center of the mixer.

4. After all AQUAFLOR® Premix has been added, allow mixer to run for ~1 minute.

5. Using a hand-held spray bottle, spray warmed fish oil as uniformly as possible over the feed.

6. After all the fish oil has been added, allow mixer to run for ~1 minute.

7. Unmixed materials, which may be trapped in the discharge valve pocket should be bled from the valve, returned to the mixer, and remixed for ~30 seconds.

8. Please refer to SOP INST 126 for cleaning and maintenance of the Marion Laboratory Mixer after each use.

APPROVED BY: _____ **DATE:** 12/10/2013
Management

APPROVED BY: _____ **DATE:** Dec 10, 2013
Study Director

Table 1. To determine amount of AQUAFLOR® Premix to add to either a 50-lb or 20-kg bag of fish feed, find the percent body weight (%BW) at which fish will be fed, go to the 4th or 5th row of the table, and read the amount (g) of AQUAFLOR® Premix to add.[a, b]

10 mg florfenicol/kg fish bw/d										
% BW	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
% AQUAFLOR	0.400	0.200	0.133	0.100	0.080	0.066	0.057	0.050	0.044	0.040
Amount of AQUAFLOR® Premix (g) to add to either a 50 lb or 20 kg bag of feed										
50 lb	90.72	45.36	30.16	22.68	18.14	14.97	12.93	11.34	9.98	9.07
20 kg	80.00	40.00	26.60	20.00	16.00	13.20	11.40	10.00	8.80	8.00

15 mg florfenicol/kg fish bw/d										
% BW	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
% AQUAFLOR	0.600	0.300	0.200	0.150	0.120	0.099	0.086	0.075	0.066	0.060
Amount of AQUAFLOR® Premix (g) to add to either a 50 lb or 20 kg bag of feed										
50 lb	136.08	68.04	45.24	34.02	27.21	22.46	19.40	17.01	14.97	13.61
20 kg	120.00	60.00	39.90	30.00	24.00	19.80	17.10	15.00	13.20	12.00

[a] Amount of AQUAFLOR® Premix (g) to add to a specific amount (g) of feed = [(amount of feed to be treated, g) × (% AQUAFLOR® Premix to add)]
[b] If the % body weight you desire is not listed on the table, refer to Section IV.A.2. for the calculation to determine % AQUAFLOR®.