

Executive Summary: Study 275.35

CVM previously conducted a study to measure the amounts and speciation of arsenic residues in tissues obtained from chickens treated with the organic arsenical, roxarsone (3-Nitro20[®]). During the course of that study multiple batches of feed were prepared; however, only one batch of feed was tested for the concentration of the active pharmaceutical ingredient. None of the batches were tested for homogeneity and stability.

This study addressed some of the issues that were identified in that study. Results from Study 275.35 were used to support a pilot study (Study 275.31) whose purpose was to generate incurred liver tissues for stability testing.

The objectives of this study were to collect samples:

- a) To measure level of arsenic in facility water;
- b) To determine the level/species distribution of arsenic (As) in the control feed;
- c) To determine the level/species distribution of As in the test article, 3-Nitro20[®];
- d) To confirm that the medicated feed has the correct concentration of roxarsone;
- e) To confirm the homogeneity of the medicated feed preparation;
- f) To determine the stability of the various arsenic species arising from addition of roxarsone Type A medicated pre-mix in the feed over an expected treatment/storage time frame of six – eight weeks.

The schedule of activities and samples collected in this study is found in Table 1.

Roxarsone medicated feed was prepared by first mixing 5.67 grams of 3-Nitro 20 (a Type A Medicated feed, which contain the roxarsone) into 5 pounds of control feed (All Grain Meat Bird Maker (crumbles), SSC-25-235107, Southern States Inc.) for 15 minutes. This mixture was then added to 45 pounds of control feed, and mixed for an additional 15 minutes. This yields a final concentration of 50 ppm of roxarsone.

Three 50 pound batches of feed were prepared. After each 50 pound batch was prepared, it was poured into a clean, large feed storage barrel. Three samples from the top, middle and bottom of each batch of medicated feed were collected and mixed to create composite samples from the top, middle and bottom. This process was repeated for the two remaining batches of roxarsone medicated feed.

Control feed (All Grain Meat Bird Maker (crumbles), SSC-25-235107, Southern States Inc.) was transferred from three 50 pound bags to a clean, large feed storage barrel. As each bag was poured into the barrel, three samples from the top, middle and bottom of each batch of medicated feed were collected and mixed to create composite samples from the top, middle and bottom. This process was repeated for the two remaining bags of control feed.

All samples were analyzed for total arsenic content and arsenic species by CVM chemists. The results are reported under Study 415.06.

TABLE 1

Step	Dates	Activity	# Samples (& Analyses)*	Sample Type**
1	18 July, 2013	Order Broiler Starter Feed (all one lot) for use here & pilot study 275.31	None	None
2	23-25 July 2013	Provide water samples from Bldg. E for analysis (3x); collect water samples for 3 days. Provide OR three (3) 1 L bottles of water.	3 (T & S)	B
	24 July 2013	Control Feed sampling: transfer 3 – 50 lb bags to storage barrel and collect samples for analyses (three composite samples per level). Provide OR all composite samples. Transfer barrel of control feed to storage room in Bldg. E.	3 (T & S)	C & P
3	24 July 2013	Sample test article(s), 3Nitro20® for analyses (3x); provide aliquots to OR.	3 (S)	P
4	26 July 2013	Prepare medicated feed using existing lot of 3Nitro20®. Provide aliquots of medicated feed samples to OR. Transfer barrel of medicated feed to storage room in Bldg. E.	3 probe (API & S) 9 mixing (T)	C & P
5	29 July 2013	Sample medicated feed – Day 3 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
6	5 August 2013	Sample medicated feed – Day 10 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
7	12 August 2013	Sample medicated feed – Day 17 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
8	19 August 2013	Sample medicated feed – Day 24 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
9	26 August 2013	Sample medicated feed – Day 31 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
10	3 September 2013	Sample medicated feed – Day 39 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
11	9 September 2013	Sample medicated feed – Day 45 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
12	16 September 2013	Sample medicated feed – Day 52 stability check; Provide aliquots of medicated feed samples to OR.	3 (API & S)	P
13	30 September 2013	Sample medicated feed – Day 66 stability check; Provide aliquots of medicated feed samples to OR. Control Feed; check to ensure lack of contamination	3 (T & S) 3 (API & S)	P

***T**=Total Arsenic; **S**=Speciation; **API**=Active pharmaceutical ingredient

****C**= composite of pour samples; **P**=samples collected using a probe; **B**=Bottle.

Composite pour samples (Top, Middle, Bottom) were obtained from each of the three bags of feed (control or medicated) that went into a single feed barrel.

For control feed, as each 50 lb feed bag was poured into the feed barrel, an aliquot was obtained from the top of the bag (Top; beginning of the pour), from the middle of the pour (Middle), and the bottom of the bag (Bottom; end of pour). A Top, Middle, and Bottom aliquot was obtained from each bag. The 3 Top, 3 Middle, and 3 Bottom aliquots were then composited by strata to create a single Top, Middle, and Bottom sample for analysis.

The same approach was used for the medicated feed. Here, the “pour” was from the feed mixing bowl at the end of the mixing period. Again, ` three batches of medicated feed were prepared, using one 50 lb feed bag per batch.

Probe samples (Top, Middle or Bottom; T, M, B) were obtained using a dedicated stainless steel probe for either the control or medicated feed. The probe had three discrete pockets to permit unique sampling at these three different strata. The barrel was divided into 4 quadrants, and four probe samples (T, M, B) were obtained at each sampling period which was composited into a single Top, Middle or Bottom probe sample.