

Executive Summary, Study 415.06

This study is part of a group of studies that were initiated by CVM to address issues raised in the review of previous work conducted by the FDA to address the safety of the arsenical drug Roxarsone. The work detailed in this summary is all the analytical work conducted to evaluate samples collected by those other studies.

Briefly, this summary describes the analytical results of water, feed, and liver tissue samples collected to address the following questions:

1. Determine the concentration of arsenic (As) in facility water
2. To analyze for homogeneity and stability of control and roxarsone-medicated feed samples;
3. Determine the concentration of total As and distribution of As species in the control feed;
4. Determine the concentration total As and distribution of As species in the test article, 3-Nitro® 20 (contains the roxarsone);
5. Verify that the medicated feed has the correct concentration of roxarsone (Rox);
6. Verify the homogeneity of the medicated feed preparation;
7. Determine the stability of the various As species arising from the addition of roxarsone Type A medicated article to the feed over an expected treatment/storage time frame of six to eight weeks;
8. Analysis of chicken liver for As species;
9. Evaluate the stability of As species to freeze and thaw cycles.

The results for total arsenic levels in the house drinking water demonstrated that the concentration was well below the limit of quantitation of 0.4 ppb. This level is well below the EPA drinking water maximum contaminant level of 10 ppb. Because our drinking water has no appreciable iAs concentration it was not the source of iAs found in chicken livers in the current study. Because the facilities water source and plumbing have not changed since the previous study was conducted, it was also likely not the source of the inorganic As in chicken livers from that study.

The levels of roxarsone and total arsenic were below the limit of quantitation.

Roxarsone-medicated feed is stable for at least 6-week feeding study. This corroborates the manufacturer's claims and also shows that degradation of medicated feed could not have been the source of inorganic As in chicken livers in the previous study conducted by CVM.

Total As concentration in treated livers from individual birds in the current study ranged from 0.6 to 1.7 ppm. Some of the individual livers comprising the composite sample may have been higher, since the composite contained 1.6 ppm total As. This corroborates the findings of the previous study where the range of total As found in the treated livers was 0.28 to 2.9 ppm. (The current tolerance is 2 ppm.).

The results the freeze and thaw cycle experiments strongly suggest that the prolonged storage of intact livers in an ultralow temperature freezer before analysis in the previous study conducted by CVM had minimal impact on those analytical findings.

The test article (3-Nitro 20) used in the current studies had higher concentrations of inorganic As than the test article used in our previous study. This meant the chickens were exposed to more inorganic As

directly in their diet in the current set of studies, which could potentially have impacted the arsenic species detected in their tissues. The most significant difference from the first study was that monomethylarsinic acid and dimethylarsinic acid were detectable (signals below the lowest validated concentration of the method) in all samples at all times in the current studies. This was most likely due to the higher concentration of As(V) in the medicated feed (88 ppb vs. 36 ppb in the first study), which in turn was due to the higher concentration of As(V) in the 3-Nitro® 20.

The Roxarsone metabolite 3-Amino is unstable in organic solution used to extract the analytes from the liver tissues. 3-Amino breaks down mostly to compounds that chromatograph after Roxarsone, with only a slight breakdown to inorganic As. This finding has minimal impact on the conclusions of our previous study.

Storage of ground liver homogenates under at refrigerator temperature for two days increases the concentrations of some As species, with 4-Arsan and As(V) increasing from undetectable concentrations, and 3-Amino increasing over 700%. This finding has no impact on the interpretation of Study 275.30, because samples were never stored at refrigerator temperature in that study. However, a brief thawing, followed by immediate refreezing at ultralow freezer temperatures has less impact on species concentrations than two days of continuous storage in a refrigerator. While the results from freeze-thaw experiment are inconclusive, they suggest that concentrations of inorganic As increase as otherwise non-extractable iAs residues are released as a function of the freeze-thaw cycle.