

Study 318.41 Executive Summary

Organic-based arsenical compounds have been used in chickens since March 21, 1944, when the drug 3-Nitro® was approved. The active ingredient in 3-Nitro® is a chemical called roxarsone. Roxarsone and other organic arsenicals (nitarsonic acid, arsanilic acid, and carbarsone) were approved for use in chickens for growth promotion, feed efficiency and improved pigmentation. The organic arsenicals, especially roxarsone, were approved in combination with other drugs such as narasin or salinomycin to prevent coccidiosis, a parasitic disease infecting the intestinal tracts in chickens which can lead to death. When 3-Nitro® (roxarsone) and the other organic arsenicals were approved, it was assumed that only organic arsenic and not inorganic arsenic would be excreted from the chickens. Organic arsenic compounds are much less toxic than inorganic arsenic, which is a known human carcinogen. Inorganic arsenic exists in two forms, arsenic (III) and arsenic (V) (pronounced arsenic-3, or trivalent arsenic, and arsenic 5, or pentavalent arsenic, respectively). The number refers to the number of electrons that elemental arsenic can donate to form other compounds. Humans (and other animals) can convert arsenic (V) to arsenic (III), which increases arsenic's toxicity and retention by the body.

In response to scientific reports that organic arsenicals could be transformed by the body into inorganic arsenic, scientists in FDA's Center for Veterinary Medicine, in collaboration with scientists from FDA's Center for Food Safety and Applied Nutrition undertook a study to address this question; can an approved organic arsenical (3-Nitro®; roxarsone) when incorporated into chicken feed and fed to chickens according to approved label directions, result in the presence of inorganic arsenic in edible tissues?

Using state-of-the art technology, FDA scientists were able to develop and validate a new analytical method that had the necessary sensitivity and specificity to detect and quantify the low levels of inorganic arsenic that were expected to be in edible tissues. The results from this work, begun in 2009, found that the livers of chickens given feed containing 3-Nitro® (Roxarsone) had concentrations of inorganic arsenic that were higher than the inorganic arsenic concentrations in the livers of chickens given control (non-medicated) feed.

A review of the results after approval of that study identified four questions that warranted additional follow-up investigation. Accordingly, three new studies were initiated to help address those questions. This report provides a unifying interpretation, summary, and conclusion for those studies

The collective goals of these studies were to answer the following questions:

Question 1. What is the homogeneity and stability of roxarsone in medicated feed?

Specifically, we were seeking to answer whether roxarsone settles over time, whether homogeneity exists in the mixed medicated feed, and whether roxarsone degrades to other arsenic species in medicated feed.

Question 2. Could the inorganic arsenic found in the livers of birds in the previous study conducted by CVM have come from a source other than the roxarsone in the medicated

feed used in that study?

Specifically, we sought to answer whether the drinking water, control feed, or contaminants in the Type A medicated article could have contributed to the inorganic arsenic found in the liver samples from CVM's previous study.

Question 3. Does the solution used to extract roxarsone and other arsenic species from tissue affect the stability of some arsenic species?

The alkaline tetramethylammonium hydroxide (TMAH) solution used in CVM's previous study was speculated to have degraded some organic arsenic species into inorganic arsenic, resulting in inorganic arsenic detection in some of the livers from chickens given roxarsone-medicated feed.

Question 4. What is the stability of roxarsone and other arsenic species when stored for prolonged periods of time at -80°C?

CVM's previous study did not assess the impact of storage time at -80°C temperatures.

Question 1. What is the homogeneity and stability of roxarsone in medicated feed? The results show that the Roxarsone Type A medicated article (specifically, 3-Nitro® 20) can be homogeneously incorporated into feed and that there was no "settling out" of roxarsone. No degradation products of roxarsone were observed during this time period. The results also demonstrated that the minimal concentrations of inorganic arsenic (Both As III and As V) were unstable and degraded over time.

These results demonstrate that the poultry would be consuming a constant amount of roxarsone in their feed throughout their entire exposure cycle. Roxarsone does not settle out of the feed to create areas with different (re: higher or lower) concentrations of roxarsone. Neither does roxarsone degrade after incorporation into feed

Question 2. Could the inorganic arsenic found in the livers of birds in CVM's previous study have come from some other source?

Analysis of the control feed showed there was no quantifiable concentration of inorganic arsenic. The results also found no quantifiable concentrations of roxarsone in the control feed. The analytical results demonstrated that the levels of arsenic in the water used for the poultry were consistently the limit of quantitation. This concentration is also well below the EPA drinking water maximum contaminant level of 10 ppb.

These results show that water is an unlikely source of the inorganic arsenic observed in the livers of poultry given roxarsone-medicated feed in CVM's previous study. Nor is their feed a likely source of the inorganic arsenic.

Question 3. Does the solution used to extract roxarsone and other arsenic species from tissue affect the stability of some arsenic species?

The Roxarsone metabolite 3-Amino was found to be unstable in organic solution used to extract the various organic and inorganic arsenic compounds from the liver tissues. The 3-Amino metabolite breaks down primarily to compounds that chromatograph after Roxarsone and inorganic arsenic (both As III and As V). There was a slight breakdown to inorganic As.

The result demonstrates that the discovery of inorganic arsenic in the livers of chickens fed roxarsone-medicated feed is due to the *in vivo* metabolism of roxarsone to produce inorganic arsenic. Thus, the presence of inorganic arsenic in these chicken livers was not a laboratory-induced artifact caused by the degradation of organic arsenic species during the process of extracting the arsenic compounds.

Question 4. What is the stability of roxarsone and other arsenic species when stored for prolonged periods of time at -80 °C?

As the results from CVM's previous study were obtained from chicken livers that had been stored for extended periods at -80 °C, there was speculation that inorganic arsenic was derived from storage-induced degradation of roxarsone. The results of these studies demonstrated that long term storage at -80 °C does not impact the stability of roxarsone or other arsenic species.

These findings strengthen the overall conclusion that the presence of inorganic arsenic species in chicken livers from poultry feed roxarsone was due to the *in vivo* metabolism of roxarsone.