Investigation into the detection of Semicarbazide (SEM), a Nitrofurazone indicator, in Chicken

Executive Summary:
An international trading partner refused entry to US chicken because it tested positive for SEM, an indicator for nitrofurazone usage. FSIS conducted an analysis to evaluate whether SEM could be detected in chicken samples collected during processing. FSIS collected and analyzed fresh and frozen (10, 20 and 30 days) chicken samples at three different points in production— at one pre-intervention point ("hot-rehang"), and two post-intervention points ("post chill" and after whole chickens have been processed to produce parts) using two different sample preparations (with and without washing steps). No samples collected at pre-intervention tested positive for SEM, whereas several post-intervention samples tested positive. SEM was detected more often, and at higher levels. While the detection of SEM in pre-intervention samples could have been indicative of nitrofurazone use, its absence in pre-intervention samples in this study suggests that the subsequent detection of SEM in the sampled products is not indicative of nitrofurazone use and may be a result of by-products formed during food processing.
1. Nitrofuran Study in Chicken Products

**Background:**
The Food Safety Inspection Service (FSIS) is the agency within the United States Department of Agriculture responsible for ensuring that the US commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled.

Nitrofuran antibiotics have been restricted from use in food-producing animals by many food regulatory agencies for many years, including: the European Commission since 1993, the U.S. Food and Drug Administration since 2002, the Thailand Ministry of Health since 2003, and the New Zealand Food Safety Authority since 2003. Those bans followed concerns about the carcinogenicity of the nitrofuran metabolites and their potential harmful effects on human health. Nitrofurazone is a nitrofuran antibiotic, and its metabolism results in tissue-bound metabolites, including semicarbazide (SEM). The furan ring is rapidly excreted, but SEM remains bound to tissue and, therefore, tissue-bound SEM has been used as a target for monitoring compliance with the restrictions on nitrofurazone use. Its usefulness as an indicator of nitrofurazone usage has been called into question as research has shown there to be alternative sources of SEM other than the use of nitrofurazone, such as environmental contaminants and reactive by-products formed during food processing. Therefore, SEM residue findings might not be indicative of nitrofurazone use, but rather a contaminant as a biproduct of non-harmful processing agents. In response to concerns raised by a foreign trading partner of the finding of semicarbazide (SEM) in raw chicken product at its port of entry, FSIS conducted a study to determine the potential source.

**Objective:**
To address the detection of SEM in chicken, FSIS conducted an exploratory study to:

1. Determine whether SEM is present in raw chicken at different points during slaughter and processing; and
2. Determine whether freezing chicken products for different lengths of time affects the detection and concentration of SEM, to mimic storage conditions during shipment to a trading partner.

**Experimental Approach:**

**Sample Collection**
FSIS collected one chicken sample, from three stages in the production process (see Figure 1), on three consecutive days, at each of the eight establishments that received a recent point of entry violation (POEV) for product testing positive for SEM. All samples were shipped to FSIS’ Western Field Laboratory for quantification of SEM.

![Figure 1: Location of sample collection in the establishment.](image-url)
Specifically, the three stages in the production process and number of samples collected were:

1. **Pre-Intervention (“Pre”) (“Hot Rehang”):**
   - Birds have been slaughtered, scalded, and de-feathered. An establishment may employ antimicrobials at points prior to evisceration as part of a multi-hurdle pathogen reduction approach. However, these were not considered interventions for the purpose of the study.
   - One carcass collected from each establishment for three consecutive days.
   - 1 sample X 3 days X 8 establishments = 24 total samples

2. **Post-Intervention (“Post”):**
   - Carcasses have undergone evisceration and proceeded through processing aimed to reduce microbial growth and prevent foodborne illness. This is the location that FSIS’ inspector typically samples carcasses for residue testing as part of the National Residue Program.
   - One carcass collected from each establishment for three consecutive days.
   - 1 sample X 3 days X 8 establishments = 24 total samples

3. **Chicken Parts (“Parts”):**
   - Carcasses have been processed (cut) into parts (leg quarters). At this stage, product has undergone all interventions and are normally packaged and stored for distribution.
   - One, 8-pound sample of chicken parts (legs or leg quarters) was collected from each establishment for three consecutive days.
   - 1 sample (8 lb. of chicken parts) X 3 days X 8 establishments = 24 total samples
   - Each 8 lb. sample was divided into four different samples at the laboratory; one was tested when fresh; three were frozen and stored for either 10, 20, or 30 days prior to SEM analysis.

Therefore, a total of 72 samples were collected and analyzed fresh. An additional 72 frozen parts samples (24 samples frozen for either 10, 20 or 30 days) were analyzed for a total of 144 samples tested.

**Laboratory Analysis**
All 144 samples were split and concurrently analyzed using FSIS’ nitrofuran method (**CLG-NFUR 3.01**), and with a modified version (removal of the multiple washing step) of that method; for a total of 288 analyses. FSIS’ nitrofuran method (**CLG-NFUR 3.01**) is based on the Food and Drug Administration’s method for the Detection of Nitrofuran Metabolites in Shrimp, but employs a multi-step chemical (alcohol) wash to remove unbound SEM prior to hydrolyzing tissue-bound SEM. The method is suitable for confirming and quantifying SEM in poultry at 0.5 μg/kg (ppb). This concentration represents the minimum level of applicability (MLA). FSIS defines an MLA as the lowest level at which a method has been successfully validated for a residue in a given matrix (meat or poultry). It also refers to the lowest level at which a laboratory analyst is expected to maintain ongoing proficiency in the method. The modified method for detecting SEM—which is referred to herein as total SEM—consisted of the **CLG-NFUR 3.01** without the multi-step chemical wash step.

**Results:**
FSIS identified multiple confirmed positive residues of SEM above the MLA (Table 1). However, all samples collected pre-intervention were negative for SEM residues. In the washed Post and Parts samples (Figure 2), regardless of whether the samples were fresh or frozen up to 30 days, the maximum level of SEM that FSIS observed was less than 2.5 ppb (Figure 3). For total SEM—that is, for samples where alcohol washes were not employed—FSIS observed a higher maximum level of SEM, with one fresh sample greater than 5 ppb (Figure 4). The highest concentration observed, just under 12 ppb, was observed for total SEM in a sample that had been frozen for 20 days (Figure 5).
Conclusions:
If SEM was indicative of nitrofurazone use in live chickens, it would have been detected in chicken samples during both pre and post application of interventions. No SEM was detected pre-intervention but was detected in samples post-intervention, indicating that the presence of SEM is not the result of nitrofurazone administration, but is likely being generated as a consequence of interventions during chicken processing. In summary, these results suggest that the SEM detected in these chicken samples is not indicative of nitrofurazone use in chicken.

Table 1: Summary of FSIS Sample Testing Results above the MLA

<table>
<thead>
<tr>
<th></th>
<th>Number of Samples above MLA (n)</th>
<th>Percentage Samples above MLA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-Intervention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SEM</td>
<td>0 (24)</td>
<td>0%</td>
</tr>
<tr>
<td>Washed</td>
<td>0 (24)</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Post-Intervention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SEM</td>
<td>22 (24)</td>
<td>92%</td>
</tr>
<tr>
<td>Washed</td>
<td>7 (24)</td>
<td>29%</td>
</tr>
<tr>
<td><strong>Packaged – Unfrozen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SEM</td>
<td>21 (24)</td>
<td>88%</td>
</tr>
<tr>
<td>Washed</td>
<td>5 (24)</td>
<td>21%</td>
</tr>
<tr>
<td><strong>Packaged – Frozen 10 Days</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SEM</td>
<td>22 (24)</td>
<td>92%</td>
</tr>
<tr>
<td>Washed</td>
<td>18 (24)</td>
<td>75%</td>
</tr>
<tr>
<td><strong>Packaged – Frozen 20 Days</strong></td>
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<td></td>
</tr>
<tr>
<td>Total SEM</td>
<td>23 (24)</td>
<td>96%</td>
</tr>
<tr>
<td>Washed</td>
<td>16 (24)</td>
<td>67%</td>
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<tr>
<td><strong>Packaged – Frozen 30 Days</strong></td>
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<td></td>
</tr>
<tr>
<td>Total SEM</td>
<td>21 (24)</td>
<td>88%</td>
</tr>
<tr>
<td>Washed</td>
<td>13 (24)</td>
<td>54%</td>
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</tbody>
</table>

*a The MLA is 0.5 ppb.
*b The results are for three samples from each establishment for each type of sample or treatment, and for each sample preparation (total SEM or with washes).
Semicarbazide Levels Detected in Chicken Samples

Figure 2: Semicarbazide levels detected in fresh (0 days) chicken samples at pre-intervention (Pre) and post-intervention (Post), and in finished parts (Parts). All samples were prepared with a multi-step chemical (alcohol) wash to remove unbound SEM prior to hydrolyzing tissue-bound SEM. Twenty-four samples were analyzed at each collection point.

Semicarbazide Levels Detected in Chicken Parts

Figure 3: Semicarbazide levels detected in fresh (0 days) and frozen chicken samples. All samples were prepared with a multi-step chemical (alcohol) wash to remove unbound SEM prior to hydrolyzing tissue-bound SEM. The analysis was conducted on fresh samples (0 days), or following freezing for 10, 20 or 30 days.
Figure 4: Total semicarbazide levels detected in fresh (0 days) chicken samples using modified-nitrofurazone method at pre-intervention (Pre) and post-intervention (Post), and in finished parts (Parts). Twenty-four samples were analyzed at each collection point.

Figure 5: Total semicarbazide levels detected in fresh and frozen chicken samples. The analysis was conducted on fresh samples (0 days), or following freezing for 10, 20 or 30 days.
References


