Presentation of David Eisenberg of Micro-Tracers, Inc. at AOAC Forum on Methods for Analysis of Antibiotics and Drugs in Feeds

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The Use of Colored Iron Particles in Determining Cross Contamination of Medicated Feeds at Feedmills and Feed Premix Plants

This topic is of current interest largely because the European Union and other major world markets are developing new food and feed regulations that are much more aggressive than what have existed in the past.

The European Union now requires that all feedmills whether they mix drugs in feeds or not must be registered with their national governments. Further, they must also have data validating the adequacy of their mixing and of their control of contamination at their feedmills and premix plants.

The issue of medicated feed contamination into non-medicated feeds is not new. In 1976, the USFDA requested the Animal Health Institute (AHI) and the American Feed Industry Association (AFIA) (then American Feed Manufacturers Association) to submit recommended "Action Levels" that could be met by industry. These were to be the maximum levels of drugs in nonmedicated feeds that would be considered acceptable or alternately the minimums that would lead to FDA enforcement actions. This AHI and AFIA did submit recommendations but opposed any action by the FDA on the matter and because of this opposition and also because the issues are complex, more than 25 years later no Action Levels exist and the FDA probably will not progress in the matter for at least a few more years.

Why is it important to minimize cross-contamination of drugs into non-medicated feeds? Such residues at high levels (i.e. 20% of formulated levels) may be toxic such as Nicarbazin reaching breeder feeds, salinomycin reaching adult turkey feeds. or monensin reaching horse feeds. Such residues at trace levels into "finisher" feeds, the last feeds fed animals prior to slaughter, may lead to illegal residues of the drugs in the tissue of the treated animals, poultry or seafood. Past problems with sulfamethazine in pork and current problems with chloramphenicol in seafood would be examples. A related concern would be the issue of ruminant by-product reaching ruminant feeds where it could lead to transmission of BSE (mad cow disease).

How is the industry currently testing to confirm manufacturing procedures are designed to minimize cross-contamination? Some manufacturers are testing for specific drugs formulated in their feeds, and using such results to establish procedures applicable to all drugs used at the feedmill.

Many drug assays are accurate and precise in detecting drug residues in meat and poultry as these are relatively simple matrices to work with. Formula feeds, however, often contain large numbers of ingredients that can make analysis for drugs at very low levels

difficult or in some cases effectively impossible. It is possible to test for cross contamination of drugs into non-medicated feed when the drug is formulated at a very high level in the medicated feed or better the premix and when the drug has a good assay at relatively low levels.

Another approach is to mix simple, easy to detect tracers most often colored iron particles into a medicated feed or premix and to determine the tracer rather than the drug, at least as a screening procedure. Currently, this approach is being actively investigated by TNO (Dutch Research Institute), Tecaliman (French Research Institute) and others, primarily in Europe.

Micro-Tracers,Inc. (San Francisco) recently participated in such a Study as described below:

Study of Cross-Contamination of Coccicor Premix 2.5% Amprolium at Feedmill/Premix Plant

Formulation of Tracers into Medicated Premix:

Two tracers, red colored iron particles with a count of 25,000/gram (micron size 150-300) and fine blue "lake" colored iron powder (micron size 50-150) were formulated at 1-kilo each per 2,000-lbs. into one 2-ton batch of Coccicor 2.5% Amprolium Premix.

Sampling of Medicated Premix and Three Subsequent Batches of Premix (pelleted) Supposed to Contain No Drug (or tracer):

From Coccicor 2.5% Amprolium Premix, one sample was taken at five locations: 1) Mixer; 2) Conveyer (after Discharge of Mixer); 3) Elevator (at top of feedmill above pelletmill); 4) Cooler (after pelleting); and 5) Packer (pellets at truck loading).

From each of the subsequent three Batches of Premix, five samples were taken from three locations: 1) Mixer; 2) Conveyer; and 3) Elevator.

The three Batches of Premix were then commingled and additional samples were taken; five samples from each of the following locations: 4) Cooler and 5) Packer.

A total of 60 samples were taken, five of the Coccicor 2.5% Amprolium Premix and fifty-five of following premixes not formulated with the drug.

Analysis of Samples:

All samples were analyzed for the red colored iron particles and for the fine blue "lake" iron powder. The tracers were isolated from the premix by magnetic separation using a "Rotary Detector" (1) equipped with a special "rare earth" magnet.

After isolating the tracers from the premix, the red tracer was demagnetized, sprinkled onto a large filter paper, sprayed with a mist of 50% ethanol with 0.5% ammonium hydroxide, the paper dried and the resulting red colored spots counted. The blue color was diffuse, not countable.

A second sub-sample was also analyzed, isolating the two tracers from the premix via magnetic separation. Instead of developing colored spots on a filter paper, the magnetic material was brushed into a centrifuge tube, diluted with 1 % sodium carbonate solution, shaken on an "auto" shaker for ten minutes, and the two colors read on a spectrophotometer.

To maximize sensitivity of the tracer methods, four gram samples of the Coccicor 2.5% Amprolium Premix were analyzed while 200 grams of all non-medicated samples were analyzed.

Twenty samples were analyzed chemically for Amprolium. These samples were chosen only after all tracer analyses had been completed. Samples were not chosen for chemical analysis at random but rather because they were thought interesting.

Tracer Results:

Batch of Coccicor 2.5% Amprolium Premix

Sample:	Weight Analyzed:	Red Count:	Red Color Absorbance:	Blue Color Absorbance:
Coccicor Mixer Sample	4 grams	121	0.28	0.42
Conveyor	4 grams	102	0.21	0.32
Top of Elevator	4 grams	85	0.14	0.25
Cooler (pellets)	4 grams	98	0.28	0.26
Coccicor (Average of samples)	4 grams	100	0.23	0.31

Batch #1 Following

Sample:	Weight Analyzed:	Red Count:	Red Color Absorbance:	Blue Color Absorbance:
Mixer (avg. five samples)	200 grams	21	0.062	0.091
Mixer Sample #1	46 grams	20	0.075	0.110
Conveyor (avg. five samples)	200 grams	395	0.597	0.675
Conveyor Sample #1	66 grams	985	2.67**	1.8**
Top of Elevator (avg. five samples)	200 grams	144	0.381	0.380
Top of Elevator Sample #1	44 grams	73	0.468	0.450

Batch #2 Following

Sample:	Weight Analyzed:	Red Count:	Red Color Absorbance:	Blue Color Absorbance:
Mixer (avg. five samples)	200 grams	0.4	Nil	Nil
Mixer Sample #1	-- grams	1	Nil	0.005
Conveyor (avg. five samples)	200 grams	47.4	0.105	0.005
Conveyor Sample #1	46 grams	104	0.212	0.255

Top of Elevator (avg. five samples)	200 grams	37	0.083	0.081
Top of Elevator Sample #1	66 grams	24	0.090	0.120

Batch #3 Following

Sample:	Weight Analyzed:	Red Count:	Red Color Absorbance:	Blue Color Absorbance:
Mixer (avg. five samples)	200 grams	1.2	Nil	0.008
Mixer Sample #1	66 grams	0	Nil	Nil
Conveyor (avg. five samples)	200 grams	19.4	0.037	0.058
Conveyor Sample #1	64 grams	59	0.122	0.220
Top of Elevator (avg. five samples)	200 grams	5.8	Nil	0.009
Top of Elevator Sample #1	44 grams	7	Nil	0.008

Batches #1, 2, and 3 Combined (Pellets)

Sample:	Weight Analyzed:	Red Count:	Red Color Absorbance:	Blue Color Absorbance:
Cooler (avg. five samples)	200 grams	36.6	0.065	0.045
Cooler Sample #1	64 grams	35	0.060	0.031
Packer (avg. five samples)	200 grams	50.8	0.059	0.045
Packer Sample #1	44 grams	105	0.100	0.058

* tracers retrieved from premix samples using Micro-Tracers, Inc. Rotary Detector laboratory magnetic separator. For color readings, diluted to 15 ml in 1% sodium carbonate solution and read at 525nm and at 630 nm, the wavelength maxima for FD&C Red#3 (erythrosine) and FD&C Blue#1 (Brilliant Blue FCF).

** for this sample only, for color reading tracer was diluted in 100 ml of 1% sodium carbonate solution with results calculated and reported to a 15 ml dilution basis.

The five samples taken from the Coccicor 2.5% Amprolium Premix yielded tracer "recoveries" as follows when compared to results for analysis of the pure tracers:

Location:	Red Particle Count:	Red Color:	Blue Color
Mixer	110%	90%	109%
Conveyer	93%	68%	84%
Top of Elevator	77%	45%	65%
Cooler (pellets)	89%	90%	69%
Packer (pellets)	84%	76%	73%
Average:	91%	74%	80%

Chemical assay results for Coccicor Amprolium 2.5% Amprolium Premix:

Location:	Amprolium:	Recovery as Compared With Specification:

Mixer-	2.02%	80%
Conveyer-	2.24%	90%
Top of Elevator-	2.14%	86%
Cooler-pellets-	2.17%	87%
Packer-pellets-	1.79%	72%
Average:	2.07%	83%

Cross Contamination of the Coccicor 2.5% Amprolium premix (average for 5 samples unless otherwise noted) into following premix production was as follows:

Location:	Red Particle Count:	Red Color:	Blue Color:
Batch #1			
Mixer	0.38%	0.40%	0.48%
Conveyor	7.2%	3.8%	3.6%
Conveyor Sample #1	17.9%	17.2%	9.5%
Top of Elevator	2.6%	2.5%	2.0%
Batch #2			
Mixer	0.01%	Nil	Nil
Conveyor	0.86%	0.68%	0.75%
Conveyor Sample #1	1.89%	1.37%	1.34%
Top of Elevator	0.67%	0.53%	0.43%
Batch #3			
Mixer	0.02%	Nil	Nil
Conveyor	0.35%	0.24%	0.30%
Top of Elevator	0.11%	Nil	Nil
Batches #1, 2, and 3 (pellets)			
Cooler	0.67%	0.42%	0.24%
Cooler Sample #1	0.64%	0.38%	0.16%
Packer	0.92%	0.38%	0.24%
Packer Sample #1	1.90%	0.65%	0.30%

* All values are percentage of originally formulated tracer not adjusted for recovery.
The chemical assay data for twenty samples can be compared with the tracer estimates of amprolium with results as follows:

Sample	Chemical Assay (ppm)	Red Tracer (ppm)	Red Tracer Color (ppm)	Blue Tracer Color (ppm)
Coccicor-2.5%	20,200	27,500	22,500	27,300
Mixer Sample	22,400	23,300	17,500	21,000
Conveyor	21,400	19,200	11,300	17,000
Top of Elevator	21,700	22,300	22,500	18,500
Cooler (pellets)	17,900	20,900	22,500	17,000
Packer (pellets)	20,700	22,600	19,300	20,200
Batch #1				
Mixer #1	370	90	120	180
Conveyor #1	4,170	4,480	4,310	2,370
Conveyor #2	1,410	1,260	1,110	1,120
Top of Elevator #1	730	790	750	590
Top of Elevator #5	600	480	390	380
Batch #2				

Mixer #1	150	Nil	Nil	Nil
Conveyor #1	660	470	340	340
Conveyor #2	170	130	160	290
Top of Elevator #1	160	110	150	160
Top of Elevator #5	170	260	270	150
Batch #3				
Mixer #1	150	Nil	Nil	Nil
Conveyor #1	520	270	200	290
Conveyor #2	180	70	30	40
Batches #1, 2, and 3 (pellets)				
Packer #1	260	480	160	80
Packer #2	210	220	70	50

Sample Calculations:

1. Red Colored Iron Particles- Particle Counts

Tracer specification (and estimated count): 25,000/gram
Formulated at 1-kilo per 2,000-lbs. of Coccicor 2.5% Amprolium premix (all 2-ton batches) Estimated particles added to premix per 2,000-lbs: 25,000 X 1,000 grams = 25,000,000. Estimated particles per four (4) gram subsample of premix: 25,000,000 divided by 2,000-lbs. divided by 454 grams X Four (4) grams = 110 particles

Actual count from Coccicor premix sample taken from Mixer: 121 (from 4 grams)
Estimated Tracer Recovery: 110% of formulated.
Estimated Amprolium: 110% X 25,000pp, specification of premix = 27,500 ppm (tracer estimate could be made more accurate by analyzing a larger sample and counting more particles)

2. Particulate Red Iron Particles- Color Readings

Absorbance found from 0.0044 grams of tracer (the amount formulated per 4 grams of Coccicor premix) diluted in 15m1 of 1% sodium carbonate in water solution = 0.309

Absorbance found from tracer from 4 grams of Coccicor premix- Mixer sample = 0.280.
Estimated Tracer Recovery: 90.3%
Estimated Amprolium: 90.3% X 25,000 ppm specification = 22,500 ppm

Absorbance found from tracer recovered from 200 grams of followup Batch #1 Conveyer #1 sample, tracer diluted in 100 ml of 7% sodium carbonate in water solution = 0.400, adjusted to dilution in 15ml = 0.400 X 6.67 = 2.70 divided by 4/200 = 0.540
Estimated Tracer Recovery = 17.5%
Estimated Amprolium: 25,000 pp, X 17.5% = 4,380 ppm

Linear regressions of this limited data yielded equations and correlation coefficients as follows:

Chemical amprolium assays with red particle counts:
Y = 220.9 +.892, correlation coefficient 0.986
Chemical amprolium assays with red color readings:
Y = 534.6 +.985 X, correlation coefficient 0.950

Chemical amprolium assays with blue color readings:
Y = 491.3 +.970 X, correlation coefficient 0.973

Conclusions:

1. The three tracer procedures - red particle counts, red and blue color readings yielded data that reflected the presence of the coded drug adequately to at least be used to screen samples for the much more expensive chemical analysis.

2. It appeared that through sequencing (no flushes were employed) cross contamination of the drug into non-medicated following feed was kept at 1% or less based on the average tracer results of final product at loadout. This low result was achieved, however, by blending three batches of following product together. If the immediately following batch had been kept isolated through the mill, cross contamination into the packed product would have probably been between 2.0% and 3.5%.

3. In testing for cross contamination at feedmills and premix plants it is critical one know and understand the flow of materials through the plant. This will allow generating meaningful information as to where and when contamination is occurring so that properly engineered solutions may be tried.

4. It is also important to know the physical properties of the medicated premix being studied, as powdered products most likely contaminate more than granulated ones though this was not evidenced by the data generated in this Study.

5. Contamination is <u>usually</u> concentrated in the first sample taken from following production. It also would increase as product flows through a manufacturing plant, though in this case the highest average levels of contamination occurred from samples taken at a conveyer exiting the surge bin.

6. Surprisingly, the red particulate tracer yielded decent colorimetric readings even from pelleted feed.

7. Interpretation of the data requires consistency. Should cross contamination results be compared with the specified level of the drug or with the amount of drug found in the formulated batch based on chemical assay of it.

8. Approximately 20 hours of laboratory time was required to generate the one-hundred and eighty tracer results generated and the value of the tracers consumed was less than $100. The cost of validating cross contamination procedures at feedmills would seem trivial though the engineering costs involved in solving problems found could be very great.

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