MIX WITH CONFIDENCE

(June 1994, International Milling Flour & Feed, page 31 to 33)

Well designed sampling and analysis procedures will prove the accuracy and homogeneity of microingredient mixing, says David Eisenberg

The mid-1990s are bringing with them an increased interest from regulatory authorities in many countries in assuring medicated feeds are mixed completely and that all microingredients are added as formulated.

If mixing is incomplete, the level of drug, in feeds will be non-uniform invalidating the safety and efficacy data used to support the use of such medications.

Failure to consistently add the formulated levels of microingredients may be a greater problem- especially in technologically advanced countries where computerized micro-bin systems are commonly used to add microingredients.

Although feed manufacturers owe a response to the public to ensure their feeds are properly manufactured. This interest coincides with a self interest to make the best product reasonably possible. This benefits the feed manufacturer's customers or the feed manufacturer himself if he is an integrated producer in the longer run, it will contribute to the survival of the manufacturer in an increasingly competitive world.

With this as background, how can the feed manufacturer validate his mixing and microingredient addition operation? The **obvious** answer is by developing and implementing well designed sampling and analysis plans to document the efficacy of these manufacturing processes.

VALIDATING THE MIXING PROCESS:

At least five issues must be considered in validating the mixing process:

1. Selection of - one or more tracers
2. Addition of the tracer to the test feed
3. Sampling the feed
4. Analysis the samples
5. Interpreting the results

Selection of the tracer

Whatever analyte is chosen for the mixer test, it will then be used as a tracer for all other ingredients. If it yields results typical of a complete mix, one will assume all other ingredients are mixed completely. This may not always be a correct assumption. Powdered feed additives may become electrostatically charged and stick to the walls of mixers that accumulate as clumps that drop off rarely. If one tests for this ingredient as a tracer, analytical results will be consistent among samples but always low on average unless one happens to test the rare clump.

Such an analyte would not accurately reflect the mixing of other ingredients and would probably be an inappropriate choice for use in evaluating mixer performance.

At least the following criteria should be considered in selecting the tracer:

A. The tracer should be contributed from only one source

B. The tracer should be a microingredient

C. There should be an analytical procedure to determine the tracer of known or determinable accuracy and precision.

D. The analytical procedure should be inexpensive

E. The analytical procedure should be quick: ideally one that may be performed "on-the spot"

F. One should be able to interpret results objectively.

Vitamin and drugs assays may be used to validate completeness of mix, but they are expensive, are often subject to considerable analytical error and often require weeks before analytical results are reported.

Salt is often used as a tracer to evaluate mixing by determining either sodium or chlorides in feeds. Salt, however, has significant deficiencies as a tracer. It is added at five to 20 kilos per metric tonne and is there by hardly a microingredient. It may not be reasonable to assume mixing of a medication added at three parts per million is correct based upon acceptable results for Salt added at 20,000 parts per million. Further, sodium and chloride may be contributed to feeds by other ingredients, yielding background "noise" confusing interpretation of results.

Minerals and amino acids are also widely used and these largely meet the criteria outlined in this paper and generally yield meaningful information. The cost of analysis for minerals such as manganese and zinc by atomic absorption spectrophotometry is often low, possibly $20/sample if preformed by a commercial laboratory or less if performed by the feed manufacturer.

Reproducibility of results on a given sample is often good with a coefficient of variation of five to eight per cent possible. Some minerals are contributed to feeds in significant quantities from only one source: the mineral premix. Amino acid analyses by HPLC may be reproducible with a coefficient of variation of five per cent or less, although they may be more costly to perform than mineral analyses.

Microtracers (tm) F (colored uniformly sized iron particles) are also used widely to evaluate mixing. These non-nutrient particulate tracers are designed to satisfy the criteria outlined in this paper. The analytical error in their determination may be two to three per cent and they may be performed "'on-the spot" by technicians with comparatively little training.

Addition of the Tracer to the Test Feed

This should be via a premix possibly made by mixing the tracer by hand with other common feed ingredient, The amount of premix added to the test feed should be similar to the lowest addition ingredient normally formulated in the feed. If medicated premixes added at 500 grams/ tons, then the tracer premix might reasonably be added at this level.

The location of tracer addition is usually where other microingredients are added. Test results will then validate existing procedure, the tracer may also be added at other locations to determine if the location of microingredient addition has a significant effect on the time required to achieve a complete mix. In several tests, it took 30 second or less to achieve a complete mix when a tracer was added into the center of the mixer is compared with when it added at the end of the mixer.

Sampling the feed

In validating mixer performance, "grab" samples should ideally be taken from within the mixer. The samples should be carefully identified and all test parameters should be carefully recorded. Samples should be adequate in size to permit repeat analysis samples evidencing unusual results.

How many samples should be taken? Taking one sample is an infinite improvement over taking none. We usually take ten samples from each of five consecutive batches and believe this adequate to detect major deviations from complete mixing that could lead to significant economic losses or regulatory compliance problems.

Analyzing the Samples

One should take a given weight of subsample from each sample for analysis without homogenizing the sample. This makes the "level of scrutiny" of the test the weight of the subsample analyzed rather than the weight of the sample taken. It makes the test more severe, increasing the likelihood of a filling conclusion but also improving the likelihood a favorable result is correct.

If mixing is good the worst of conditions, then it should be good under less severe ones. The ideal "level of scrutiny" would be batches of feed consumed by target animals poultry or fish at one feeding. The ideal "level of scrutiny" for the tracer would be one added at the lowest level of any microingredient formulated in the feed, though this issue is complex and effected by intermediate mixing procedures utilized to achieve adequate dispersion. For example, critical microingredients may be dissolved in a liquid and sprayed onto a dry carrier to facilitate achieving adequate dispersion.
Samples should then be analyzed using appropriate methodology, proper instrumentation and skilled personnel.

Interpreting Results

This should be done by comparing the coefficient of variation found from the test data with the coefficient of variation inherent in the method. The method coefficient of variation is what one would expect from repeat analysis of the same sample.

Using a maximum permitted coefficient of variation of 10 per cent is arbitrary and capricious unless it can be related to the variability of the analysis. If a coefficient of variation of two per cent can be achieved from a method, this could be used as the goal for judging a mix complete. If a coefficient of variation of 15 per cent was the best that could be achieved front a method, this could be used as a goal for judging a mix complete. Such standards would be unrealistically high however, as 50 per cent of all tests of a complete mix would evidence more variation than the goal.

A more realistic goal would be to consider results acceptable if they would occur by chance from a complete mix in at least one per cent of all tests of such a mix. This may mean considering data as evidencing a compete mix when it yields a coefficient of variation is 50 per cent or more greater than the method coefficient of variation.

In evaluating particulate tracer counts, one may utilize Poisson statistics to determine if mixing is complete. If one makes no allowance for analytical error, if a mix is complete it should yield test results with 1 standard deviation equal to the square root of the average count. An average count of 100 should yield a standard deviation of 10 and a coefficient of -variation of 10 per cent. An average count of nine would yield an expected standard deviation of three and an expected coefficient of variation of 33 per cent.

VALIDATING Batching

This can be done by evaluating the data generated front the mixer test. The total amount of tracer found in each batch may be compared and in the case of particulate tracers interpreted statistically. If statistically the same amount of tracer is found in each batch, this data will support efficacy of the batching operation.

MICROTRACER DATA FROM TWO TESTS:

Data from a test evidencing complete mixing and consistent addition of tracer to a series of four batches of feed. Blue Tracer Counts

Data from a test evidencing incomplete mixing and a failure to add the same amount of tracer to a series of five batches. Red Tracer Counts

Blue Tracer Counts

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Total
1	93	101	109	99	402
2	105	96	98	104	403
3	122	95	103	111	431
4	117	96	113	103	429
5	98	106	106	103	413
6	102	115	95	102	414
7	108	103	116	104	431
8	111	98	100	87	396
9	98	115	116	107	430
10	98	110	88	109	405
Total	1,052	1,035	1,044	1,029	4,060
Prob. Of Occurring by Chance	49.5%	74.0%	48.1%	87.5%	-
Coefficient of Variation	8.8%	7.7%	9.0%	6.6%	-

Probability The Total Counts By Location Would Occur By Chance Front a Complete Mix: **79.8%**

Probability. The Total Counts By Batch Would Occur By Chance From a Complete Mix: **86.3%**

Red Tracer Counts

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Total
1	84	76	91	90	85	426
2	68	74	83	90	56	371
3	56	101	105	117	94	473
4	79	122	129	115	60	505
5	52	108	140	122	96	518
6	102	120	106	116	94	538
7	146	95	101	106	50	498
8	126	95	129	140	75	565
9	96	94	96	111	61	458
10	66	95	118	128	56	463
Total	875	980	1,098	1,135	727	4,815
Prob. Of Occurring by Chance	0.0%	0.32%	0.05%	1.42%	0.01%	-
Coefficient of Variation	34.9%	16.2%	18.5%	15.5%	18.2%	-

Probability The Total Counts by Location Would Occur By Chance Front a Complete Mix: **0.01%, (1 in 10,000 tests)**

Probability The Total Counts by Batch Would Occur By Chance From a Complete Mix: **0.07% (7 in 10,000 tests)**

June 1994, International Milling Flour & Feed