PRODUCTSCHAP DIERVOEDER

GMP+ Certification Scheme for the Animal Feed Sector 2006

Company Minimum Requirements

for Sampling and Analysis

Appendix 4

Adopted by the Central College of Experts for the Animal Feed Sector	1 July 2009
Approved by the Executive Committee of the Product Board Animal Feed	--
Publication / Version	20 October 2009 (corr. 09 November 2009)
Effective date:	20 October 2010

Table of Contents

PART A: SAMPLING AND ANALYSIS PROTOCOLS

2.5 TESTING PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER IN PREMIX AND ADDITIVES INSTALLATIONS

1. SYSTEM

The method of measurement of carry-over in premix and additives installations corresponds as far as the systematics are concerned to Chapters 2.2 to 2.4.

2. CARRY-OVER PROCESS

- The carry-over process to be measured relates to the point where the additives and/or animal veterinary products are added to the bulk vehicle load or the bag filling.
- Measurement of the carry-over should be carried out for each production line in the installation.
- The measurement should be carried out with a quantity of mix which is equal to the smallest batch which in practice may be produced on the production line in question.

3. TRACER SUBSTANCE TO BE USED

The following tracer substance can be used for the measurement of carry-over:
cobalt mixes in accordance with Chapter 2.2 or 2.3.4 with a cobalt concentration of at least 200 mg/kg. At cobalt concentrations of 2,000 mg/kg or more use may also be made of pure cobalt sulphate. In addition the microtracers FSS-Lake and F-Lake and methyl violet can be used in the dosage of 10 mg/kg. Otherwise there should be compliance with Chapter 2.3.4.

4. DETERMINATION OF CARRY-OVER

The measurement of carry-over is done by taking the mix in which the carry-over occurs into consideration as a whole. This means that the average level in this mix is the departure point for determining the carry-over. The carry-over is measured as follows:

a. mix the whole mix again
b. take and analyse 5 samples from this mix (V1 to V6). The average level is calculated from this
c. The carry-over is measured as follows:

$$\frac{\text{(average quantity in mix in which carry-over occurs)}}{\text{(batching in previous mix from which there is carry-over)}} \times 100\%$$

2.6 CHECKING PROCEDURE FOR THE PROCESS ACCURACY OF COMPOUND FEEDS WITH MICROTRACERS

1. FIELD OF APPLICATION

This testing procedure or method for the determining of the homogeneity of meals and grains may be used on the usual premixes and mixes of ground compound feed raw materials in compound feed companies.

The method can also be used to obtain an indication of the carry-over percentage which occurs in compound feed raw materials.

2. DEFINITIONS

Production plant: A production plant is an installation which is suitable for the preparation of compound feeds.

Microtracer mix: For the testing of a compound feed the microtrace mix contains 4 kg feed lime or wheat grits and 100 g microtracer. Therefore 100 g microtracer is mixed with 1 t compound feed, which corresponds to a mixing accuracy of 1:10 000.
For the testing of a premix the microtrace mix contains 4 kg feed lime or wheat grits and 10 g microtracer. Therefore 10 g microtracer is mixed with 1 t compound feed, which corresponds to a mixing accuracy of 1:100 000.

3. PRINCIPLE

So-called microtracers are used as a measuring substance. These are elementary iron particles which are coated with a feed colourant in order to be able to count the colour points in the analysis. An average number of particles per mg is indicated in the analysis certificate for the microtracer used. For the microtracer particles it is a case of particle distribution thus the average number of particles varies depending on the microtracer batch. In order to determine the number of particles in question in the test a microtracer mix is produced in which the average number of particles is determined exactly for the microtracer used (see 17).

Two different microtracers are suitable for the homogeneity and carry-over analysis. These are distinguished by their particle size and therefore the number of particles per mg. Microtracer F consists of particles with a size distribution of 150 – 300 µm and have been used for some time in the feed industry. The somewhat finer microtracer FSS with a size distribution of 75 – 150 µm was specially developed for chicken feeds to decrease the test quantity used.

The required accuracy for the determination of carry-over of 1% is achieved in both microtracer F and FSS. In order to achieve a statistically accurate assessment, a minimum number of 15 particles must be present per filter. Only then can an accurate assessment of homogeneity be made for the first production batch.

Method	Average number of particles per milligram [mg]	Test quantity for the assessment of homogeneity [g]	Average expected number of particles in the tested quantity	Test quantity for determination of carry-over [g]	Accuracy of the carry-over examination in %	Average expected number of particles in the tested quantity
FSS-Lake 100 ppm	200	2	40	200	1	40
F-Lake 100 ppm	25	20	50	2000	1	50
FSS-Lake 10 ppm	200	25	50	2500	1	50

Table 1:

The control procedure for the determination of the degree of homogeneity of meal mixes in the preparation of compound feeds makes use of a microtrace mix which, with respect to its properties, can replace the usual compound feed additives.

The control procedure includes the processing of two batches from the same feed mix. The microtrace mix (see section 2) is added to the first batch. The number of particles of microtracer in the samples of meal and grain from the first batch of feed is then determined. The second production batch consists of the bare feed without the microtracer mix. The microtracer level of the meal and grain samples from this batch is also determined. This level gives a picture of the carry-over which is taking place in the production installation.

The number of particles of microtracer in the samples which were taken is determined by separating the microtracer particles from the other feed particles using a rotary detector and by using the feed colourant to make the separate microtracer particles visible on a sheet of filter paper.

4. EQUIPMENT AND TOOLS

The following are required for the carrying out of the testing procedure:

- 40 plastic sample bags for keeping the samples of meal and grain each with a capacity of twice the quantity of the sample (see table 2)
- 40 plastic sample bags for keeping the samples of meal and grain each with a capacity of twice the quantity of the sample (see table 2)
- one small and one large plastic scoop for taking the samples.

The number of bags specified is required if production plant samples of meal are taken at one point in the production installation and samples of grains are taken at another point. For each subsequent sampling point 40 bags extra are needed.

Method	Sample quantity to be taken from production batch 1 for the determination of homogeneity	Sample quantity to be taken from production batch 2 for the determination of carry-over
FSS-Lake 100 ppm	≥ 4 g	≥ 400 g
F-Lake 100 ppm	≥ 40 g	≥ 4,000 g
FSS-Lake 10 ppm	≥ 50 g	≥ 5,000 g

Table 2:

A laboratory must be available where microtracer analyses can be done. Appointments should be made in good time with this laboratory for analyses to be carried soon after the samples are taken.

5. COMPANY DETAILS REQUIRED

The following will be requested in advance from a compound feed company at which a control procedure is to be carried out:

a. a block diagram of the production installation in which it can be indicated during the implementation where the microtracer mix has been added and where samples are taken.

The following will be requested during the implementation of the control procedure:

b. the computer prints or copies of them which show:
   - the composition of the feed mix
   - the batch weight requested by the computer, and
   - the actual batch weight
   - or, if there is no computerisation:
   - the composition of the feed mix
   - the calculated batch weight This weight is obtained by adding the weights of the components
   - the read-out of the actual batch weight.

The following will be requested to be able to calculate the batch weight for the mixer and the grain press:

c. where and how much molasses, vinasse and other liquid ingredients added to the main flow of the feed, and
d. where and how much fats, etc., are added to the main flow. The requested addition points are shown in the block diagram.

6. ADDITION OF THE MICROTRACER MIX

The microtrace mix (see section 2) is added to the first batch. The place where the microtrace mix is added depends on the carry-over path to be measured (see 7.1). The place where the addition of the microtrace mix must be done should be indicated in the block diagram for the production plant.
The place where the addition of the microtrace mix must be done should be indicated in the block diagram for the production plant. The batch weight requested by the process computer may be assumed.

7. TAKING AND HANDLING SAMPLES

7.1 Analysis samples

7.1.1 Taking the samples

During the implementation of the control procedure in a compound feed company samples are taken at locations agreed in advance:

- after the mixer but as close as possible to the mixer (see 13.1)
- from the entrance to the finished product silo in the event of meal production or a pressed meal silo
- from the entrance to the finished product silo in the event of grain production
- another desired end point for the determination of the relevant carry-over path

If the meal or grain flow is not reachable at the desired locations then suitable openings should be made in consultation with the company.

Twenty samples are taken each time per sampling point. The statistical certainty is increased through the rise in the number of samples. The increase in the number of samples from 30 to 40 is, however, voluntary.

Meal production
From the first batch 20 samples of meal (immediately after the mixer) and 20 samples of meal (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

From the second batch 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

Grain production
From the first batch 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis.

From the second batch of feed 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

If a split is desired with respect to the carry-over by the dosage/grinding/mixing line on the one hand and the press line on the other hand then during the first and second batches another 20 samples of meal for microtracer determination should be taken at the input to the pressed meal silo. The method of working is identical to the method for meal production.

Sample bags
All sample bags are provided with a sample code before the start of the production of the first batch of feed. The sample bags must be filled up to the edge and sealed air-tight to avoid demixing (in the case of meal samples) as much as possible.

Sampling

1. Production batch: Once the meal and/or grains flow starts for the batch to be inspected then 20 samples of meal and 20 samples of grains are taken spread as well as possible over the duration of the batch.

2. Production batch: Due to the irregular distribution to be expected of the microtracer particles in the carry-over batch (in the beginning very high numbers of microtracer particles and at the end very low numbers of microtracer particles) the sampling is done in a different way. The first three samples are continuously collected in a large collection container. The first sample represents the sampling time from 0 to 0.5 min, the second sample 0.5 to 1.0 minutes and the third sample 1.0 to 1.5 minutes in the feed flow. A sample is taken from each of these three collection samples via sampling splitting (quartering method). The other samples are taken as random samples every 0.5 minutes. For a total duration of the feed of 10 minutes there will be 20 samples collected of which the first three are collective samples and the other 17 are individual samples. For lesser durations the sampling intervals must be modified accordingly.

N. B.: It is very important that the samples are taken spread as well as possible over the duration of the batch in connection with the samples being representative of the batch as a whole.

9. PROCESSING OF THE RESULTS

9.1 Non-standard results

After the addition of the microtracer mix to the feed in the first batch the microtracer level in the first samples to be taken will be lower than in the subsequent samples. This is because of a degree of carry-over of bare feed from the feed batch prior to the batch with microtracer.

An opposite effect is seen in the samples from the second batch of feed. Now the first samples show a relatively high microtracer level as a result of carry-over of feed containing microtracer from the second to the third batch. Normally the spread of the microtracer levels in the samples from the third batch is considerably more distorted than in the second batch. There is also no calculation of a probability for homogeneity and it is enough to make a graph of the average microtracer level per sample against the sample number. In as far as the samples are properly representative for the whole batch which means they have been properly spread over the total duration, the average carry-over of microtracer can be calculated as a percentage of the microtracer level in batch one.

9.2 The carry-over

The carry-over for the installation is calculated as follows in accordance with this control procedure per measurement point.
The average microtracer level of the analysis samples from the second batch divided by the average microtracer level on the basis of dry matter from the analysis samples from the second batch. By multiplying this figure by 100 the average carry-over percentage can be calculated.

9.3 The test for homogeneity

The following statistical data will be determined for the evaluation:

- average number of particles
- standard deviation for the number of particles
- $\chi 2$ (chi squared) – value
- Probability p in % as an indication of the homogeneity
- Microtracer recovery percentage in %.

The probability is determined using the determined chi squared value and the number of degrees of freedom (see table 3). Values between 0.999 and < 0.0005 can be found. The assessment of the homogeneity is recorded by definition. The probability is calculated using an Excel table.

7.1.2 Preparation of the samples
Each meal and grain sample is ground in a suitable grinder.

First grind the samples of meal and grain from the second batch (carry-over batch) and then those from the first batch. This ensures that the samples are ground in ascending order of their microtracer level.

Clean the grinder after each sample using compressed air.

Clean the grinder after each group of 20 samples using both compressed air and, after disassembly of the relevant parts, by brushing clean with a brush which is not too soft. There may be no carry-over of material from the previous group of samples.

Homogenise each grinding as much as possible and then place it back in the original bag.

7.1.3 Storage of analysis samples
Analysis samples which will not be tested within a week of being taken should be stored dry.

7.2 Analysis of samples
The sample packaging may not be opened during this period (see 13.2).
Homogenise the mix to be inspected in the sample bag as much as possible by stirring it with a spoon or spatula.
A sample of the desired size is taken from the sample to be analysed and subjected to a microtracer analysis.

7.3 Archiving
The filters with the colour points from the individual microtracer particles must be archived. A minimum archiving period of 1 year is suitable. The filter sheets can, however, be retained for more than 10 years.

8. DETERMINATION OF THE MICROTRACER PARTICLES

The microtracer particles from a sample are isolated because of their magnetic properties by way of filtering through a rotary detector with a rotary magnet. Other magnetic particles are also filtered out at the same time. The identification of the microtracer particles takes place by way of a bonding colouring agent which causes a chromatographic effect (= colour point) on a filter sheet after treatment with a developer. In order to make the colour points visible the filter is dampened with the developer, the microtracer particles are transferred quantitatively to the filter sheet and the colour development is stopped by then laying the filter sheet on a heated plate.

Other magnetic particles do not develop colour points and are removed from the filter sheet with a brush. The colour points developed on the filter sheet are counted. The microtracer level is indicated as the number of particles per gram of sample.

χ^2	1	2	3	4	5	6	7	8	9
1	.317	.607	.801	.910	.963	.986	.995	.998	.999
2	.157	.368	.572	.736	.849	.920	.960	.981	.991
3	.083	.223	.392	.558	.700	.809	.885	.934	.964
4	.046	.135	.261	.406	.549	.677	.780	.857	.911
5	.025	.082	.172	.287	.416	.544	.660	.758	.834
6	.014	.050	.112	.199	.306	.423	.540	.647	.740
7	.008	.030	.072	.136	.221	.321	.429	.537	.637
8	.005	.018	.046	.092	.156	.238	.333	.433	.534
9	.003	.011	.029	.061	.109	.174	.253	.342	.437
10	.002	.007	.019	.040	.075	.125	.189	.265	.350
11	.001	.004	.012	.027	.051	.088	.139	.202	.276
12	.001	.002	.007	.017	.035	.062	.101	.151	.213
13	**	.002	.005	.011	.023	.043	.072	.112	.163
14	**	.001	.003	.007	.016	.030	.051	.082	.122
15	**	.001	.002	.005	.010	.020	.036	.059	.091

Table 3: Table for the determination of probability, horizontal: number of degrees of freedom, vertical: chi squared values

10. REPORTING

The following is reported for each group of feed samples:

1. For the calculation of the homogeneity of the first batch of compound feed, the average number of microtracer particles in whole numbers
2. For the calculation of the homogeneity of the first batch of compound feed, the number of degrees of freedom of the system Number of analysed samples n-1
3. for the calculation of the homogeneity in the first batch of compound feed, the chi squared value (calculated from the empiric coefficient of variation for the analysed samples times the number of data divided by the average number of particles in the analysed samples)
4. from the number of degrees of freedom and the chi squared value, the probability as a percentage of the analysed samples [[(Chivert (chi squared; degree of freedom) × 100] × 100]
5. the calculated recovery percentage of the microtracer particles in the first batch of feed in relation to the number of microtracer particles in the added microtracer mix
6. The calculated carry-over in the installation from the number of microtracer particles in the second batch of feed in relation to the number of microtrace particles in the first batch

11. ASSESSMENT OF THE RESULTS

Homogeneity of the material

The calculated probability as a percentage is a measure for the homogeneity of the meal mix or grains in question from which the samples were taken. The probability indicates how probable it is that the tested sampled corresponds to a perfect mix.

If the value found in the test is identical with a probability of more than 5 % (0.05) then it may be assumed on the basis of the probability calculation that there is a "perfect mix".
If the value found in the test is identical with a probability of between 1% and 5% (0.01 to 0.05) then it may be assumed on the basis of the probability calculation that there is a "probable significant deviation from a perfect mix". This refers to a borderline case about which no unambiguous statement can be made. The test must be repeated.
If the value found in the test is identical with a probability of less than 1% then it may be assumed on the basis of the probability calculation that there is a "probable significant deviation from a perfect mix".
A key feature of the poisson distribution is that when there is a "perfect mix" the standard deviation of a test series must be (on average) equal to the square root of the average.

Two examples follow of the calculation of a homogenous and a non-homogenous mix.

Example 1: Homogeneous mix

Sample number	Number of particles counted, x	Average m	Difference x_n-d_n	Square of difference d_n^2
1	47	50	3	9
2	53	50	3	9
3	45	50	5	25
4	55	50	5	25
5	50	50	0	0
Average x=50			Sum d_n^2=S=68	

Table 4: Example of the calculation for a homogenous mix

Number of samples: n=5
Chi squared value χ^2: S: x = 1 (68: 50 = 1.4)
Table values from table 3:
horizontal: n – 1 = 4
vertical: 1
calculated probability:0.910
calculated probability in %: 91.0%

Result: The calculated probability is greater than 5 %; there is therefore a homogenous mix.

Example 2: Non-homogenous mix

Sample number	Number of particles counted, x	Average m	Difference x_n-d_n	Square of difference d_n^2
1	43	53	10	100
2	57	53	4	16
3	70	53	17	289
4	35	53	18	324
5	61	53	8	64
Average x=53			Sum d_n^2=S=793	

Table 5: Example of the calculation for a non-homogenous mix

Number of samples: n=5
Chi squared value χ^2: S: x = 15 (793: 53 = 15)
Table values from table 3:
horizontal: n – 1 = 4
vertical: 15
calculated probability:0.005
calculated probability in %: 0.5%

Result: The calculated probability is less than 1 %; there is therefore a non-homogenous mix.

12. NOTES

12.1 First sampling point
A feed mix is not homogenous after the dosage of the various components. Even after the grinding of the raw materials in the hammer mill this is only partly the case. Often finer raw materials are led around the hammer mill and carried straight to the mixer. A homogenous feed mix may therefore only be expected for the first time in the mixer. Taking samples directly from the mixer is difficult and may be dangerous and is certainly not recommended. The sampling should therefore be done after the mixer. In most companies this will be the outflow of the bunker under the mixer.

12.2 Storage of the samples
Samples which can not be examined in the short term should be stored in a dry area to retain sufficient free-flow for the test.

13. SAFETY

The control procedure is usually carried out in practice in a compound feed company.

For those who carry out the control procedure in a compound feed company the following safety rules apply:

a. the operatives will make themselves aware before the start of the work of the safety instructions which apply in the compound feed company
b. during their stay in the compound feed company the operatives are bound to follow the safety instructions of the compound feed company

14. PROCESSING OF COMPOUND FEED CONTAINING MICROTRACER

No special instructions.

15. LITERATURE

1. The use of Microtracers to determine Completeness of Mix
The use of microtracers for the determination of the homogeneity of mixes
David A. Eisenberg, President of Micro Tracers, Inc. of San Francisco

2. Mix with Confidence
Safe mixing
David A. Eisenberg, President of Micro Tracers, Inc. of San Francisco
International Milling Flour&Feed, June 1994

2.7 CONTROL PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER USING MICROTRACERS BY WEIGHING

1. FIELD OF APPLICATION

See 2.6 Control procedure for the measurement of carry-over using microtracers

2. DEFINITIONS

Production plant: A production plant is an installation which is suitable for the preparation of compound feeds.

Microtracer mix: For the testing of a compound feed the microtrace mix contains 4 kg feed lime or wheat grits and 500 g microtracer. Therefore 500 g microtracer is mixed with 1 ton of compound feed, which corresponds to a mixing accuracy of 1: 2000.

3. PRINCIPLE

Use will be made for the measuring substance of the so-called RF microtracer (elementary iron particles). With an average number of particles of 1,000,000 per gram. For the microtracer particles it is a case of particle distribution; the average number of particles varies depending on the microtracer batch. In order to determine the number of particles in question in the test a microtracer mix is produced in which the average number of particles is determined exactly for the microtracer used.

The number of particles of microtracer in the samples which were taken is determined by separating the microtracer particles from the other feed particles using a rotary detector. The sample should be led twice over the rotary detector for this.

Once the sample has passed the magnet then the excess product is brushed from the filter with a brush, do this accurately and with a rotating magnet.
Remove the filter from the magnet and transfer and return the microtracer in a tared copper weighing boat.

NB 1: In order to correct for "factory iron" at least three blank samples will be measured. In the final calculation there should be a correction for the average of the blank measurements.

NB 2: When adding the microtracer mix there should be 500 gram/ ton added. The sample size should be 300-500 grams.

4. EQUIPMENT AND TOOLS

See 2.6 Control procedure for the measurement of carry-over using microtracers

5. COMPANY DETAILS REQUIRED

See 2.6 Control procedure for the measurement of carry-over using microtracers

6. ADDITION OF THE MICROTRACER MIX

See 2.6 Control procedure for the measurement of carry-over using microtracers

7. TAKING AND HANDLING SAMPLES

See 2.6 Control procedure for the measurement of carry-over using microtracers

8. DETERMINATION OF THE MICROTRACER PARTICLES

By way of double filtration using a rotation detector with a rotary magnet the microtracer particles from a sample are isolated because of their magnetic properties. Other magnetic particles are also filtered out at the same time.
The identification of the microtracer particles is done by weighing.

NB 1: In order to correct for "factory iron" at least three blank samples will be measured. In the final calculation there should be a correction for the average of the blank measurements.
NB 2: When adding the microtracer mix there should be 500 gram/ ton added. The sample size should be 300-500 grams.

9. PROCESSING OF THE RESULTS

See 2.6 Control procedure for the measurement of carry-over using microtracers

10 REPORTING

See 2.6 Control procedure for the measurement of carry-over using microtracers

11. ASSESSMENT OF THE RESULTS

See 2.6 Control procedure for the measurement of carry-over using microtracers

12. REMARKS

See 2.6 Control procedure for the measurement of carry-over using microtracers

13. SAFETY

See 2.6 Control procedure for the measurement of carry-over using microtracers

14. PROCESSING OF COMPOUND FEED CONTAINING MICROTRACER

See 2.6 Control procedure for the measurement of carry-over using microtracers

2.8 CONTROL PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER IN ANIMAL FEED PREPARATION USING METHYL VIOLET

This text will be added later.

2.6 *CHECKING PROCEDURE FOR THE PROCESS ACCURACY OF COMPOUND FEEDS WITH MICROTRACERS*

1. FIELD OF APPLICATION

This testing procedure or method for the determining of the homogeneity of meals and grains may be used on the usual premixes and mixes of ground compound feed raw materials in compound feed companies.

The method can also be used to obtain an indication of the carry-over percentage which occurs in compound feed raw materials.

2. DEFINITIONS

Production plant:	A production plant is an installation which is suitable for the preparation of compound feeds.
Microtracer mix:	For the testing of a compound feed the microtrace mix contains 4 kg feed lime or wheat grits and 100 g microtracer. Therefore 100 g microtracer is mixed with 1 t compound feed, which corresponds to a mixing accuracy of 1:10 000. For the testing of a premix the microtrace mix contains 4 kg feed lime or wheat grits and 10 g microtracer. Therefore 10 g microtracer is mixed with 1 t compound feed, which corresponds to a mixing accuracy of 1:100 000.

3. PRINCIPLE

So-called microtracers are used as a measuring substance. These are elementary iron particles which are coated with a feed colourant in order to be able to count the colour points in the analysis. An average number of particles per mg is indicated in the analysis certificate for the microtracer used. For the microtracer particles it is a case of particle distribution thus the average number of particles varies depending on the microtracer batch. In order to determine the number of particles in question in the test a microtracer mix is produced in which the average number of particles is determined exactly for the microtracer used (see 17).

Two different microtracers are suitable for the homogeneity and carry-over analysis. These are distinguished by their particle size and therefore the number of particles per mg. Microtracer F consists of particles with a size distribution of 150 – 300 µm and have been used for some time in the feed industry. The somewhat finer microtracer FSS with a size distribution of 75 – 150 µm was specially developed for chicken feeds to decrease the test quantity used.

The required accuracy for the determination of carry-over of 1% is achieved in both microtracer F and FSS. In order to achieve a statistically accurate assessment, a minimum number of 15 particles must be present per filter. Only then can an accurate assessment of homogeneity be made for the first production batch.

Method	Average number of particles per milligram [mg]	Test quantity for the assessment of homogeneity [g]	Average expected number of particles in the tested quantity	Test quantity for determination of carry-over [g]	Accuracy of the carry-over examination in %	Average expected number of particles in the tested quantity
FSS-Lake 100 ppm	200	2	40	200	1	40
F-Lake 100 ppm	25	20	50	2000	1	50
FSS-Lake 10 ppm	200	25	50	2500	1	50

Table 1:

The control procedure for the determination of the degree of homogeneity of meal mixes in the preparation of compound feeds makes use of a microtrace mix which, with respect to its properties, can replace the usual compound feed additives.

The control procedure includes the processing of two batches from the same feed mix. The microtrace mix (see section 2) is added to the first batch. The number of particles of microtracer in the samples of meal and grain from the first batch of feed is then determined. The second production batch consists of the bare feed without the microtracer mix. The microtracer level of the meal and grain samples from this batch is also determined. This level gives a picture of the carry-over which is taking place in the production installation.

The number of particles of microtracer in the samples which were taken is determined by separating the microtracer particles from the other feed particles using a rotary detector and by using the feed colourant to make the separate microtracer particles visible on a sheet of filter paper.

4. EQUIPMENT AND TOOLS

The following are required for the carrying out of the testing procedure:

- 40 plastic sample bags for keeping the samples of meal and grain each with a capacity of twice the quantity of the sample (see table 2)
- 40 plastic sample bags for keeping the samples of meal and grain each with a capacity of twice the quantity of the sample (see table 2)
- one small and one large plastic scoop for taking the samples.

The number of bags specified is required if production plant samples of meal are taken at one point in the production installation and samples of grains are taken at another point. For each subsequent sampling point 40 bags extra are needed.

Method	Sample quantity to be taken from production batch 1 for the determination of homogeneity	Sample quantity to be taken from production batch 2 for the determination of carry-over
FSS-Lake 100 ppm	≥ 4 g	≥ 400 g
F-Lake 100 ppm	≥ 40 g	≥ 4,000 g
FSS-Lake 10 ppm	≥ 50 g	≥ 5,000 g

Table 2:

A laboratory must be available where microtracer analyses can be done. Appointments should be made in good time with this laboratory for analyses to be carried soon after the samples are taken.

5. COMPANY DETAILS REQUIRED

The following will be requested in advance from a compound feed company at which a control procedure is to be carried out:

a. a block diagram of the production installation in which it can be indicated during the implementation where the microtracer mix has been added and where samples are taken.

The following will be requested during the implementation of the control procedure:

b. the computer prints or copies of them which show:

- the composition of the feed mix
- the batch weight requested by the computer, and
- the actual batch weight
- or, if there is no computerisation:
- the composition of the feed mix
- the calculated batch weight This weight is obtained by adding the weights of the components
- the read-out of the actual batch weight.

The following will be requested to be able to calculate the batch weight for the mixer and the grain press:

c. where and how much molasses, vinasse and other liquid ingredients added to the main flow of the feed, and

d. where and how much fats, etc., are added to the main flow. The requested addition points are shown in the block diagram.

6. ADDITION OF THE MICROTRACER MIX

The microtrace mix (see section 2) is added to the first batch. The place where the microtrace mix is added depends on the carry-over path to be measured (see 7.1). The place where the addition of the microtrace mix must be done should be indicated in the block diagram for the production plant.
The place where the addition of the microtrace mix must be done should be indicated in the block diagram for the production plant. The batch weight requested by the process computer may be assumed.

7. TAKING AND HANDLING SAMPLES

7.1 Analysis samples

7.1.1 Taking the samples

During the implementation of the control procedure in a compound feed company samples are taken at locations agreed in advance:

- after the mixer but as close as possible to the mixer (see 13.1)
- from the entrance to the finished product silo in the event of meal production or a pressed meal silo
- from the entrance to the finished product silo in the event of grain production
- another desired end point for the determination of the relevant carry-over path

If the meal or grain flow is not reachable at the desired locations then suitable openings should be made in consultation with the company.

Twenty samples are taken each time per sampling point. The statistical certainty is increased through the rise in the number of samples. The increase in the number of samples from 30 to 40 is, however, voluntary.

Meal production
From the first batch 20 samples of meal (immediately after the mixer) and 20 samples of meal (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

From the second batch 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

Grain production
From the first batch 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis.

From the second batch of feed 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

If a split is desired with respect to the carry-over by the dosage/grinding/mixing line on the one hand and the press line on the other hand then during the first and second batches another 20 samples of meal for microtracer determination should be taken at the input to the pressed meal silo. The method of working is identical to the method for meal production.

Sample bags
All sample bags are provided with a sample code before the start of the production of the first batch of feed. The sample bags must be filled up to the edge and sealed air-tight to avoid de-mixing (in the case of meal samples) as much as possible.

Sampling

1. Production batch: Once the meal and/or grains flow starts for the batch to be inspected then 20 samples of meal and 20 samples of grains are taken spread as well as possible over the duration of the batch.

2. Production batch: Due to the irregular distribution to be expected of the microtracer particles in the carry-over batch (in the beginning very high numbers of microtracer particles and at the end very low numbers of microtracer particles) the sampling is done in a different way. The first three samples are continuously collected in a large collection container. The first sample represents the sampling time from 0 to 0.5 min, the second sample 0.5 to 1.0 minutes and the third sample 1.0 to 1.5 minutes in the feed flow. A sample is taken from each of these three collection samples via sampling splitting (quartering method). The other samples are taken as random samples every 0.5 minutes. For a total duration of the feed of 10 minutes there will be 20 samples collected of which the first three are collective samples and the other 17 are individual samples. For lesser durations the sampling intervals must be modified accordingly.

N. B.: It is very important that the samples are taken spread as well as possible over the duration of the batch in connection with the samples being representative of the batch as a whole.

7.1.2 Preparation of the samples
Each meal and grain sample is ground in a suitable grinder.

First grind the samples of meal and grain from the second batch (carry-over batch) and then those from the first batch. This ensures that the samples are ground in ascending order of their microtracer level.

Clean the grinder after each sample using compressed air.

Clean the grinder after each group of 20 samples using both compressed air and, after disassembly of the relevant parts, by brushing clean with a brush which is not too soft. There may be no carry-over of material from the previous group of samples.

Homogenise each grinding as much as possible and then place it back in the original bag.

7.1.3 Storage of analysis samples
Analysis samples which will not be tested within a week of being taken should be stored dry.

7.2 Analysis of samples
The sample packaging may not be opened during this period (see 13.2).
Homogenise the mix to be inspected in the sample bag as much as possible by stirring it with a spoon or spatula.
A sample of the desired size is taken from the sample to be analysed and subjected to a microtracer analysis.

7.3 Archiving
The filters with the colour points from the individual microtracer particles must be archived. A minimum archiving period of 1 year is suitable. The filter sheets can, however, be retained for more than 10 years.

8. DETERMINATION OF THE MICROTRACER PARTICLES

The microtracer particles from a sample are isolated because of their magnetic properties by way of filtering through a rotary detector with a rotary magnet. Other magnetic particles are also filtered out at the same time. The identification of the microtracer particles takes place by way of a bonding colouring agent which causes a chromatographic effect (= colour point) on a filter sheet after treatment with a developer. In order to make the colour points visible the filter is dampened with the developer, the microtracer particles are transferred quantitatively to the filter sheet and the colour development is stopped by then laying the filter sheet on a heated plate.

Other magnetic particles do not develop colour points and are removed from the filter sheet with a brush. The colour points developed on the filter sheet are counted. The microtracer level is indicated as the number of particles per gram of sample.

9. PROCESSING OF THE RESULTS

9.1 Non-standard results

After the addition of the microtracer mix to the feed in the first batch the microtracer level in the first samples to be taken will be lower than in the subsequent samples. This is because of a degree of carry-over of bare feed from the feed batch prior to the batch with microtracer.

An opposite effect is seen in the samples from the second batch of feed. Now the first samples show a relatively high microtracer level as a result of carry-over of feed containing microtracer from the second to the third batch. Normally the spread of the microtracer levels in the samples from the third batch is considerably more distorted than in the second batch. There is also no calculation of a probability for homogeneity and it is enough to make a graph of the average microtracer level per sample against the sample number. In as far as the samples are properly representative for the whole batch which means they have been properly spread over the total duration, the average carry-over of microtracer can be calculated as a percentage of the microtracer level in batch one.

9.2 The carry-over

The carry-over for the installation is calculated as follows in accordance with this control procedure per measurement point.
The average microtracer level of the analysis samples from the second batch divided by the average microtracer level on the basis of dry matter from the analysis samples from the second batch. By multiplying this figure by 100 the average carry-over percentage can be calculated.

9.3 The test for homogeneity

The following statistical data will be determined for the evaluation:

- average number of particles
- standard deviation for the number of particles
- $\chi 2$ (chi squared) – value
- Probability p in % as an indication of the homogeneity
- Microtracer recovery percentage in %.

The probability is determined using the determined chi squared value and the number of degrees of freedom (see table 3). Values between 0.999 and < 0.0005 can be found. The assessment of the homogeneity is recorded by definition. The probability is calculated using an Excel table.

χ^2	1	2	3	4	5	6	7	8	9
1	.317	.607	.801	.910	.963	.986	.995	.998	.999
2	.157	.368	.572	.736	.849	.920	.960	.981	.991
3	.083	.223	.392	.558	.700	.809	.885	.934	.964
4	.046	.135	.261	.406	.549	.677	.780	.857	.911
5	.025	.082	.172	.287	.416	.544	.660	.758	.834
6	.014	.050	.112	.199	.306	.423	.540	.647	.740
7	.008	.030	.072	.136	.221	.321	.429	.537	.637
8	.005	.018	.046	.092	.156	.238	.333	.433	.534
9	.003	.011	.029	.061	.109	.174	.253	.342	.437
10	.002	.007	.019	.040	.075	.125	.189	.265	.350
11	.001	.004	.012	.027	.051	.088	.139	.202	.276
12	.001	.002	.007	.017	.035	.062	.101	.151	.213
13	**	.002	.005	.011	.023	.043	.072	.112	.163
14	**	.001	.003	.007	.016	.030	.051	.082	.122
15	**	.001	.002	.005	.010	.020	.036	.059	.091

Table 3: Table for the determination of probability, horizontal: number of degrees of freedom, vertical: chi squared values

10. REPORTING

The following is reported for each group of feed samples:

1. For the calculation of the homogeneity of the first batch of compound feed, the average number of microtracer particles in whole numbers
2. For the calculation of the homogeneity of the first batch of compound feed, the number of degrees of freedom of the system Number of analysed samples n-1
3. for the calculation of the homogeneity in the first batch of compound feed, the chi squared value (calculated from the empiric coefficient of variation for the analysed samples times the number of data divided by the average number of particles in the analysed samples)
4. from the number of degrees of freedom and the chi squared value, the probability as a percentage of the analysed samples [[(Chivert (chi squared; degree of freedom) × 100] × 100]
5. the calculated recovery percentage of the microtracer particles in the first batch of feed in relation to the number of microtracer particles in the added microtracer mix
6. The calculated carry-over in the installation from the number of microtracer particles in the second batch of feed in relation to the number of microtrace particles in the first batch

11. ASSESSMENT OF THE RESULTS

Homogeneity of the material

The calculated probability as a percentage is a measure for the homogeneity of the meal mix or grains in question from which the samples were taken. The probability indicates how probable it is that the tested sampled corresponds to a perfect mix.

If the value found in the test is identical with a probability of more than 5 % (0.05) then it may be assumed on the basis of the probability calculation that there is a "perfect mix".
If the value found in the test is identical with a probability of between 1% and 5% (0.01 to 0.05) then it may be assumed on the basis of the probability calculation that there is a "probable significant deviation from a perfect mix". This refers to a borderline case about which no unambiguous statement can be made. The test must be repeated.
If the value found in the test is identical with a probability of less than 1% then it may be assumed on the basis of the probability calculation that there is a "probable significant deviation from a perfect mix".
A key feature of the poisson distribution is that when there is a "perfect mix" the standard deviation of a test series must be (on average) equal to the square root of the average.

Two examples follow of the calculation of a homogenous and a non-homogenous mix.

Example 1: Homogeneous mix

Sample number	Number of particles counted, x	Average m	Difference x_n-d_n	Square of difference d_n^2
1	47	50	3	9
2	53	50	3	9
3	45	50	5	25
4	55	50	5	25
5	50	50	0	0
Average x=50			Sum d_n^2=S=68	

Table 4: Example of the calculation for a homogenous mix

Number of samples: n=5
Chi squared value χ^2: S: x = 1 (68: 50 = 1.4)
Table values from table 3:
horizontal: n – 1 = 4
vertical: 1
calculated probability: 0.910
calculated probability in %: 91.0%

Result: The calculated probability is greater than 5 %; there is therefore a homogenous mix.

Example 2: Non-homogenous mix

Sample number	Number of particles counted, x	Average m	Difference x_n-d_n	Square of difference d_n^2
1	43	53	10	100
2	57	53	4	16
3	70	53	17	289
4	35	53	18	324
5	61	53	8	64
Average x=53			Sum d_n^2=S=793	

Table 5: Example of the calculation for a non-homogenous mix

Number of samples: n=5
Chi squared value χ^2: S: x = 15 (793: 53 = 15)
Table values from table 3:
horizontal: n – 1 = 4
vertical: 15
calculated probability: 0.005
calculated probability in %: 0.5%

Result: The calculated probability is less than 1 %; there is therefore a non-homogenous mix.

12. NOTES

12.1 First sampling point

A feed mix is not homogenous after the dosage of the various components. Even after the grinding of the raw materials in the hammer mill this is only partly the case. Often finer raw materials are led around the hammer mill and carried straight to the mixer. A homogenous feed mix may therefore only be expected for the first time in the mixer. Taking samples directly from the mixer is difficult and may be dangerous and is certainly not recommended. The sampling should therefore be done after the mixer. In most companies this will be the outflow of the bunker under the mixer.

12.2 Storage of the samples

Samples which can not be examined in the short term should be stored in a dry area to retain sufficient free-flow for the test.

13. SAFETY

The control procedure is usually carried out in practice in a compound feed company.

For those who carry out the control procedure in a compound feed company the following safety rules apply:

a. the operatives will make themselves aware before the start of the work of the safety instructions which apply in the compound feed company
b. during their stay in the compound feed company the operatives are bound to follow the safety instructions of the compound feed company

14. PROCESSING OF COMPOUND FEED CONTAINING MICROTRACER

No special instructions.

15. LITERATURE

1. The use of Microtracers to determine Completeness of Mix
The use of microtracers for the determination of the homogeneity of mixes
David A. Eisenberg, President of Micro Tracers, Inc. of San Francisco

2. Mix with Confidence
Safe mixing
David A. Eisenberg, President of Micro Tracers, Inc. of San Francisco
International Milling Flour&Feed, June 1994

2.7 CONTROL PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER IN ANIMAL FEED PREPARATION USING METHYL VIOLET

This text will be added later.