Guidance

S9.14 - Examples of methods for measuring carry-over and homogenity

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1. Introduction

This support document contains detailed descriptions of methods for measuring carry-over and homogeneity and can also be used for measuring carry-over and homogeneity of critical feed additives and veterinary medicinal products.

Despite the supporting character of this document the wording MUST is used frequently. This does not mean that you must use these methods, but using the word 'must' is inherent to a method description: if you choose to use this method, you must follow the instructions described.

It is recommended to perform a test to confirm that a mixture containing critical feed additives / veterinary medicinal products is homogeneous before performing the carry-over test.

2. Testing procedure for the carry-over in compound feed mixing using a mix of manganese oxide and a protein-rich and a protein-poor mix

2.1. Application area

The testing procedure was developed for the determination of the carry-over which occurs in compound feed production companies.

By collecting the samples at various different places in the production process, insight can be obtained into the carry-over in the production installation (for example: grinding / mixing line to pressed meal bunker or the press / cooling line). The method is also suitable for the determination of the extent to which homogeneous mixes can be produced using the installation (see § 2.8).

2.2. Principle of the testing procedure

The testing procedure is carried out by first fabricating a protein and Mn-rich Soya mix and immediately afterwards by fabricating a protein and Mn-poor mix on the same production line. The increase in the protein and Mn level of the maize mix during the running of the production line is caused by carry-over. By relating this increase to the protein and Mn level of the Soya mix, the carry-over level can be calculated.

Because the protein and manganese oxide content of the maize mix progresses hyperbolically (from high levels at the beginning of the flow to lower levels afterwards), the sampling procedure must be given particular attention.

2.3. Equipment and Tools

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

a. a quantity of manganese oxide corresponding to 0.4% of the usual batch size
b. (possibly) a scoop for taking samples
c. two buckets to be able to collect a number of sub-samples
d. sample pots or bags which can hold at least 200 grams of material. If the carry-over measurement is carried out at two places in the production line then 20 sample pots will usually be enough (only 14 samples will actually be tested).

2.4. Company details required

The following must be known about the company where the testing procedure will be carried out:

a. the flowchart of the production installation

b. the way in which the Soya and maize mix is put together. An exact indication must in particular be given of how and where the manganese oxide is added and how any transport system for the manganese oxide to the mixer is flushed both for the Soya mix and for the maize mix.

2.5. Implementation of the testing procedure

2.5.1. Fabrication of the protein and Mn-rich Soya mix

The Soya mix (with the usual batch size) consists of 92% Soya meal, 4% fat/oil, 3% cane molasses, 0.4% manganese oxide and 0.8% dicalcium phosphate (or calcium carbonate or salt). This mixture is batched, ground, mixed and pelletised in the usual way. Molasses and fat/oil are added to obtain a meal with normal physical characteristics which can be pelletised properly. The Soya meal may come from more than one batching silo.

The manganese oxide comes instead of the premix and must take the same route as the premix. The manganese oxide is therefore batched into the premix weighing machine or dumping pit.

The batching must be carried out such that the manganese oxide comes virtually fully to the bottom of the premix weighing machine or dumping pit.

The manganese oxide must comply with the following requirements:

1. Mn level at least 50%
2. particle size: 100% must be smaller than 0.2 mm.

Normally, calcium carbonate, salt and/or feed phosphate is batched via the same weighing machine or dump pit. Because of this the carry-over of components from the premix will be less especially when first the premix and only then the other products are batched.

For the testing procedure first 0.4% manganese oxide and then 0.8% calcium carbonate, feed phosphate or salt is batched.

Once the content of the premix weighing machine (or the dumping pit) has been added to the Soya mix in the mixer, the normal mixing time is carried out. The mix is then removed to an empty pressed meal bunker and pelleted (sample).

The grinding/mixing line and the press/cooling line may not be used for anything other than the maize mix after the Soya mix.

2.5.2. Sampling of the Soya mix

When unloading the Soya pellets in the finished product silo a good mix sample is taken from the last part of the batch.

2.5.3. Fabrication of the protein and Mn-poor maize mix

The maize mix (with the same batch size as the Soya mix) consists of 92% maize, 4% fat/oil, 3% cane molasses and 0.8% dicalcium phosphate (or calcium carbonate or salt). If it is not possible to batch 92% maize then a maize/wheat mix or another protein-poor mix may be put together (sample).

The transport system between the premix weighing machine (or dumping pit) and the mixer is flushed with 0.8% dicalcium phosphate (or salt or calcium carbonate).

The mixing time starts once the feed phosphate has been added to the mix. The mix is then removed to the (empty) pressed meal bunker (sample) and then pelletised (sample).

2.5.4. Sampling of the maize mix

The following samples of the maize mix are collected:

1. the maize (and possibly the wheat) which is used for the composition of the mix
2. six samples from the maize mix at the inflow to the pressed meal bunker
3. six samples from the maize mix at the inflow to the final product silo.

The sampling procedure is important for the samples in 2. and 3. In particular the first part of the meal or the pellets from the batch will have higher levels of protein and manganese oxide which will then decrease relatively quickly to a lower and more constant level. It is therefore important to sample the first part of the meal or pellet flow intensively and to know to which part of the feed these samples relate.

The sampling procedure at the inflow to the pressed meal bunker (which usually lasts three to five minutes) is as follows:

1. during the first 30 seconds as many sub-samples as possible are collected in a bucket; a mix sample is made from these
2. for the second 30 seconds: idem
3. then every 30 seconds a random sample from the flow is collected until the meal flow stops.

The total running time of the meal flow is noted and six samples are kept, namely the three which were taken first and three of the other samples.

The sampling of the pellets at the inflow to the finished product silo takes place in the same way. Because the total duration is usually somewhat longer the procedure is now as follows:

1. during the first minute as many sub-samples as possible are collected in a bucket; a mix sample is made from these
2. during the second minute: idem
3. then every minute a random sample from the flow is collected until the pellet flow stops.

Note: If the pellet flow is not continuous then the "real" duration must be used.

Note the total duration here as well and keep six samples, namely the three which were taken first and three of the other samples.

2.5.5. Processing of the Soya mix in compound feed

At low carry-over levels the Soya mix has a Mn level of c. 2,000 mg/kg. In the processing of this Soya mix in compound feed account must be taken of the fact that the Mn level of compound feed may be a maximum of 250 mg/kg.

2.6. The analysis of the samples

In total there are 14 (or possibly 15) samples collected:

1 sample of Soya pellets (+ Mn) = A
1 sample of maize (pure) (+ possible wheat) = B
6 samples of maize mix meal (pressed meal bunker) = C (1 to 6)
6 samples of maize mix meal (finished product silo) = D (1 to 6)
All samples are analysed for CP and Mn.

Half of the samples of maize meal mix and maize mix pellets are analysed for moisture; this is in order to find out whether the moisture content has changed during pelletising. If the moisture content has clearly changed during pelletising then the CP and Mn levels of the maize mix pellets must be corrected for the moisture content of the maize mix meal.

2.7. The calculation of the carry-over percentages

The carry-over percentages can be calculated from the levels of CP and Mn in the samples taken. Suppose that the following levels are found:
Soya pellets: 420 grams CP and 2,006 mg Mn/kg
Pure maize: 86 grams CP and 4 mg Mn/kg
samples maize mix (above the pressed meal bunker)
1. mix sample (0.5 min.)160 grams CP and 400 mg Mn/kg
2. mix sample (0.5 min.) 100 grams CP and 60 mg Mn/kg
3. random sample 90 gram and 27 mg
4. random sample 85 grams (avg. 88) and 30 mg (avg. 28)
5. random sample 88 gram and 28 mg
6. random sample 89 gram and 27 mg
The total duration of the meal flow in the pressed meal bunker = 5.5 min.
Expected levels of maize mix (92% maize and 3% molasses with 40 grams CP and 25 mg Mn/kg):
CP = 0,92* 86 + 0,03* 40 = 80,3 gram/kg
Mn = 0,92* 4 + 0,03* 25 = 4,4 mg /kg
The average levels of CP and Mn in the maize mix are calculated as follows:
CP = 0,5/5,5* 160 + 0,5/5,5* 100 + 4,5/5,5* 88 =95.6 grams/kg
Mn = 0,5/5,5* 400 + 0,5/5,5* 60 + 4,5/5,5* 28 = 64.7 mg/kg
(samples 1 and 2 each have a duration of 0.5 minutes from a total duration of 5.5 minutes. For samples 3 to 6 the average level is calculated; the duration of this is
5.5 – 2 x 0.5 = 4.5 minutes).
The carry-over percentage (Vs-%) is now calculated as follows:

$$Vs\text{-}\% = \frac{\text{avg. level in maize mix} - \text{expected level in maize mix}}{\text{avg. level in Soya pellets} - \text{expected level in maize mix}} \times 100$$

The carry-over percentages are then (up to the pressed meal bunker)

for CP = ((95,6 - 80,3) / (420 - 80,3)) * 100 = 4.5%

and for Mn = ((64,7 - 4,4) / (2.006 - 4,4)) * 100 = 3%

- The carry-over percentages at the inflow to the finished product cell are calculated in the same way.
- The carry-over percentage of the CP relates to the feed as such, from the batching equipment.
- The carry-over percentage for the Mn gives an indication of the carry-over of components from the premix.

2.8. The measurement of homogeneity

In order to determine the extent to which the installation produces homogeneous mixes, at least 10 samples must be collected from the Mn-rich Soya mix and analysed for Mn. The spread of the Mn levels of these samples (standard deviation or the difference between the highest and lowest value) is a measure of homogeneity.

When taking the samples from the Soya mix one must ensure that the whole flow of the mix is sampled. Because it is often not known exactly how long the meal flow will last, it is desirable in the first instance to take a generous number of samples of which only a part (namely 10) need to be tested.

The homogeneity test may be carried out at many places in the installation. If the samples are taken immediately after the mixer then a good picture is obtained of the functioning of the mixer.

If, on the other hand, samples are taken at other places in the installation (but after the mixer) then the homogeneity will generally be less than immediately after the mixer.

This is because in this case de-mixing and carry-over also play a role. Because the Mn-rich Soya mix is always produced after a “normal” compound feed with much lower Mn levels, the first samples of the Soya mix will be contaminated with a certain amount of compound feed and will therefore contain less Mn. The subsequent samples will be contaminated with less and less normal compound feed and will have higher and higher Mn levels.

2.9. Errors discussion

Table 1 shows which Mn and protein levels are to be expected in the maize mix at the various carry-over percentages, assuming 80 grams CP and 5 mg Mn/kg maize mix (pure) and 400 gram CP and 1,800 mg Mn/kg Soya mix.

Table 1 Effect of carry-over percentage on Mn and protein level of the maize mix						
Carry-over %	0	1	3	5	10	15
Mn from basis*	5	5	5	5	5	5
From Soya	0	18	54	92	180	270
	5	23	59	95	185	275
* effect of thinning discounted						
CP from basis	80	79,2	77,6	76	72	68
From Soya	0	4	12	20	40	60
	80	83,2	89,6	96	112	128

On the basis of the analysis accuracy of the Mn and CP determination an estimate can be made of the accuracy with which the carry-over percentage can be determined.

For the six maize samples to be tested it is assumed that the average Mn-level found in 95% of the cases will lie between 95 and 105% of the actual level; for levels < 60 mg/kg the absolute interval is made equal to the interval for 60 mg/kg, thus +/- 3 mg/kg.

For the Soya mix it is assumed that the Mn level found in the analysis will deviate by a maximum of 100 mg/kg from the actual level.

For the protein it is assumed that the average level found for the six maize samples will in 95% of cases lie between 99 and 101% of the actual level and that the level found for the Soya mix will deviate by a maximum of 2% from the actual level.

The results of the calculations are shown in Table 2.

It may be concluded that low carry-over percentages can be determined fairly reliably. For low carry-over levels Mn seems to comply better than the CP; at high carry-over levels, on the other hand, the protein gives better results than the Mn.

Table 2: Effect of the analysis accuracy on the carry-over percentage to be established				
		Maize mix		
Carry-over level		Calculated	Interval analysis	Carry-over percentage*
Mn	0 1 3 5 10 15	5 mg/kg 23 59 95 185 275	2 - 8 mg/kg 20 - 26 56 - 62 90 - 100 176 - 194 261 - 289	0,16 - 0,18% 0,8 - 1,2 2,7 - 3,4 4,5 - 5,6 9 - 11,1 13,5 - 16,7
* On the basis of 1800 mg Mn/kg Soya mix (variation 1700-1900, at low Mn in maize there is a calculation of high Mn in Soya, and vice versa).				
		Calculated	Interval analysis	Carry-over %*
CP	0 1 3 5 10 15	80 g/kg 83,2 89,6 96 112 128	79.2 - 80.8 g/kg 82,4 - 84,0 88,7 - 90,5 95,0 - 97,0 110,9 - 113,1 126,7 - 129,3	- 0,25 - 0,25 0,7 - 1,3 2,6 - 3,4 4,5 - 5,5 9,4 - 10,6 14,2 - 15,8
* On the basis of 400 g CP/kg Soya mix (variation 392-408, at low CP in maize there is a calculation of high CP in Soya, and vice versa).				

3. Testing procedure for the measurement of homogeneity and carry-over in installations for premixtures, feed additives and compound feed with microtracers

3.1. Field of application

This procedure may be used in the feed production industry for determining the homogeneity in premixtures, feed additives and compound feed or any other particle mixture. With an appropriate pre-treatment it is also applicable to a wide range of matrices like pelleted feed or extruded feed.

This procedure can also be used to determine the carry-over to subsequent batches.

3.2. Definitions

Microtracer particles: Very fine particles with a high iron content coated with a nontoxic food colourant (e.g. Microtracer- Lake particles) The colour is not visible in feed and is treated during analysis to develop the colour.
F particles: Microtracer particles with a mean of 25.000 particles per gram.
FS particles: Microtracer particles with a mean of 50.000 particles per gram
FSS particles: Microtracer particles with a mean of 500.000 particles per gram.
Microtracer premix: Preparation of Microtracer particles and limestone or other appropriate carriers. It is used to apply the Microtracer to the feed production line in the same way micro-ingredients of the test batch are added in the production plant. Each Microtracer premix comes from the producer with a certificate of analysis.
Rotary Detector: Rotating permanent magnetic tool used to quantitatively separate small magnetic particles.

3.3. Principle

Two subsequent batches have to be tested to check homogeneity and carry-over. Microtracers are added to the first batch only. They are added to the feed production line like other micro-ingredients. The usual feed composition and production procedure don´t have to be modified for the test. Care has to be taken that no additional Microtracer (e.g. for marking) is contained in the added premix. To determine homogeneity samples are taken directly after the mixer and from each final feed (e.g. meal and/or pellets) at the end of the production line. For carry-over measurements samples are taken from the second feed batch to which no Microtracer has been added. The samples are analysed for Microtracer content by separating the magnetic particles with a rotating permanent magnetic tool, the rotary detector. To distinguish between Microtracers and other magnetic particles the colour of the Microtracer particles is made visible and countable using chromatography.

The number of Microtracer particles monitors directly the quality of the mixing and the amount of carry-over, respectively. Both batches can be used as feed because Microtracer particles are nontoxic and do not colour the feed.

Extra clarification: Even strong magnets do not necessarily have to be turned off for testing as they may lower the recovery rate but do not influence the distribution of the Microtracer.

3.4. Company details required

The following information will be requested in advance:

1. a flowchart of the production installation to note where the Microtracer premix is added and where the samples are taken
2. expected batch size
3. appropriate carrier for preparation of Microtracer premix

The following information will be requested during sampling:

4. computer prints or copies which show:
   a. the composition of the feed mix
   b. the batch size requested by the computer
   c. the actual batch size according to the batch protocol
5. or, if there is no computer:
   a. the name and article number of the feed mix
   b. the calculated batch size (obtained by adding the weight of all components)
   c. the read-out of the actual batch size.

The following information will be requested to be able to calculate the batch size for the mixer and the batch size of the final product.

6. weight and addition point of liquid ingredients (molasse, vinasse, etc.)
7. weight and addition point of fats/oils etc.
8. the addition points have to be noted in the flowchart

3.5. Planning of the test

Before sampling the test has to be planned in detail. In case of small batch sizes (below 100 kg) the pure Microtracer FSS can be added, in case of bigger batch sizes it is added as a premix. The concentration and amount of Microtracer premix have to be chosen to allow later during analysis to count 100 - 200 particles per sample on one filter paper.

For the preparation of the premix the following calculations are necessary:

3.5.1. Homogeneity (batch 1)

<u>Dosing of the Microtracer particles:</u>

Information required:

a. accuracy to be checked (e.g. 1:100 000)
b. size of Microtracer premix
c. batch size of the test mix

d. number of Microtracer particles per gram (from certificate of analysis)

Calculations:
a. Weight of pure Microtracer to be added:
   batch size × accuracy = weight of Microtracer to be incorporated in Microtracer premix.
   The complete Microtracer premix is added to the first batch. (A small amount is kept for analysis when preparing the premix.)
b. The total number of added Microtracer particles is calculated:
   weight of Microtracer × number of Microtracer particles per gram = number of Microtracer particles added
c. theoretical concentration of Microtracer particles in feed of first batch:
   number of particles added / batch size = amount of Microtracer particles per gram feed

Example:
1. accuracy to be checked: 1: 100 000
2. weight of added Microtracer premix: 4000 g
3. batch size of the test mix 1000 kg = 1 000 000 g
4. Microtracer FSS has about 500 000 particles per gram

Calculations:
a. Microtracer weight to be added: 1 000 000 g × 1:100 000 = 10 g.
   A Microtracer premix is prepared with 10 g Microtracer FSS and 3990 g limestone (or a different suitable carrier).
b. Total number of particles: 10 × 500 000 = 5.000.000 particles. The complete Microtracer premix is added to the first 1000 kg test batch in the feed production.
c. theoretical concentration in first batch: 5 000 000 / 1 000 000 g = 5 particles per gram feed.

Sample size for Microtracer analysis:
The sample size for each Microtracer analysis is chosen to yield 100 – 200 particles per filter paper.

Example:
In the given example samples of 20 g must contain 20 g × 5 particles per g =100 particles which can be counted easily on one filter paper.

Sampling from the production line:
To determine homogeneity, samples from batch 1 are taken directly after the mixer or if technically impossible directly from the mixer and from each final feed at the end of the production line. At each sampling place ca. 20 samples (e.g. after the mixer HM1 – HM20 and from the final product HF1 – HF20) are taken spread as well as possible over the duration of the batch.

The sample size must allow analysing each sample at least three-times. Usually a 100 g sample will be sufficient.

3.5.2. Carry-over (batch 2)

No addition of Microtracer particles:

To check the carry-over no Microtracer particles are added to the second, subsequent batch. This batch must follow the very same way through the production line (e.g. same silos, same transportation belts) as batch 1 of homogeneity. The carry-over level of Microtracer particles from the first batch is measured.

Sample size for Microtracer analysis:
Usually very low amounts of Microtracer particles are expected. About 400 – 1000 g of each sample is analysed. As the highest carry-over is expected in the first three samples the samples C 1 – C 3 (see section 9) are analysed in appropriate fractions to avoid overload of the filters (max. 200 particles).

Sampling from the production line:
Ca. 20 samples (C1 – C20) are taken from each final feed at the end of the production line evenly spread over the whole flow-time. The carry-over is expected to be higher in the first samples and very low in the end. Usually a sample size of 400 – 1000g is sufficient.

3.5.3. Further sampling places

If further sampling places are requested, sampling must be planned according to the purpose of the measurement in line with the principles laid down under 5.1 and 5.2.

Tracer and concentration	Size of sample for homogeneity (from batch 1)	Size of sample for carry-over (from batch 2)
FSS 10 ppm	ca. 100g	ca. 400-1000g
FS 100 ppm	ca. 100g	*ca. 400 - 1000g*
F 100 ppm	ca. 100g	ca. 400-1000g

3.6. Equipment and tools

To take samples at the production plant the following is needed:
- for homogeneity testing: ca. 40 small plastic sampling bags (200 ml), provided with a sample code
- for carry-over testing: ca. 20 large plastic bags (2000 ml), provided with a sample code
- for each extra sampling place: ca. 20 plastic bags (volume depends on expected Microtracer concentration), provided with a sample code
- adequate sampling tools (e.g. small and large scoop for taking the samples in the bags)

To analyse the Microtracer content:
- see section 9

3.7. Sampling from the production line

The Microtracer premix is obtained in the concentration planned in section 5 and added to the mixer in the same way micro-ingredients are added during the production process (e.g. micro dosing silo, directly into the mixer, or via hand tipping into the mixer). Samples are taken as planned (see section 5) and stored almost air-tight in sampling bags.

Sampling has to be retained as documented information in a sampling protocol, comprising:

a. date of sampling
b. name of personnel who does the sampling
c. batch details (see section 5)
d. number of samples
e. place, where samples are taken
f. sample codes
g. any other relevant information

Samples are stored dry at room temperature (if there are no special requirements) and transferred to the laboratory in due time.

3.8. Preparation of samples

If the samples taken are not in meal form (e.g. pelleted or extruded feed) the samples have to be ground in a suitable grinder (e.g. Retsch mill, 1mm sieve).

The samples have to be ground in order of increasing expected Microtracer content, i.e. starting with the last samples of batch 2. In batch 1 the sequence of grinding is not crucial, because all samples must contain the same amount of Microtracer particles.

Clean the grinder thoroughly after each sample: use compressed air, disassemble relevant parts, sweep with a brush or a hand broom and/or use a vacuum cleaner. No carry-over of material from previous samples is allowed.

3.9. Determination of Microtracer particles

Equipment:
1. Rotary Detector
2. Demagnetizing equipment
3. Gloves
4. Paper and pencil
5. Appropriate vessel and tablespoon for weighing
6. Scale
7. Small filter paper, diameter: 70 mm
8. Large filter paper, diameter: 180 mm or bigger e.g. DIN A4
9. fan brush
10. Basin for developing solution
11. appropriate absorptive paper
12. tweezers
13. Heating plate (90°C)

Chemicals:
developing solution: 7 % sodium carbonate solution.

Sequence of the analysis:
In the laboratory the samples are analysed in the order of expected increasing number of Microtracer particles, i.e. from C20 to C1 and from H1 to H20 (the order is not relevant here).

Sample amount for assay:

1. carry-over:

For the analysis of carry-over the sample amount analysed must be about 400 g to 1000 g. The lower the expected carry-over level is, the higher the sample amount must be.

Example: About 800 g to 1000 g samples must be analysed for an expected carry-over level below 1%. To find the right sample weight analyse 500 g of a sample from the middle of the feed flow (e.g. sample C10). Count the particles and adapt the weight, so that if possible in minimum 30 particles are counted. If necessary, weigh less (may be half, i.e. 250 g) for the first three samples with the highest expected carry-over, because the particle count must not exceed 200 particles per filter. For installations with a very low expected carry-over the particle count per sample may be below 30.

2. homogeneity:

The sample amount has been estimated in section 4. To check if this is the right sample weight, analyse 20 g of a sample from the middle of the feed flow (e.g. sample H10). Count the particles and adapt the weight, so that 100 - 200 particles per filter are counted. Analyse approximately this weight for all samples from the homogeneity batch. Do not weigh exactly this weight, generally weigh two tablespoons and note the exact weight.

Execution of the analysis:

a. Gloves must be used during analysis.
b. Place a small filter paper on the magnet in the Rotary Detector and replace the top hopper.
c. Weigh the amount of sample to be assayed. Note the weight.
d. Turn on the Rotary Detector (normal operation, see instruction manual Rotary Detector).
e. Transfer the sample completely into the Rotary Detector using a clean brush.
f. Remove the top hopper of the Rotary Detector (Auto-stop operation: the rotating magnet stops automatically)
g. Turn on the Rotary Detector for the so-called “brushing mode” (the Rotary Detector works for five seconds and then stops automatically again). Within these five seconds clean the small filter paper and the edge of the fixation ring from light substances of the feed (mainly fine dust particles), using a brush.
h. Wet the large filter paper completely in the developing solution basin, put the filter paper on a clean smooth work surface and absorb excess developing solution with paper
i. Remove the fixation ring from the magnet and carefully transfer the small filter paper straight upwards from the rotary magnet without losing Microtracer particles
j. Demagnetize the Microtracer particles on the small filter paper: hold the small filter paper above the demagnetizer at a distance of about one cm, turn on the demagnetizer with the other hand, move the small filter paper straight upwards without turning off the demagnetizer, afterwards turn off the demagnetizer
k. Transfer the small filter paper horizontally above the large filter paper
l. Sprinkle the Microtracer particles from the small filter paper to the large filter paper, so that all particles lie separate: for this purpose touch the Microtracer particles on the small filter paper with one finger and move the small filter paper slowly above the large filter paper to spread the particles over the large filter paper with this finger. Turn the small filter paper and tap the back side of the small filter paper to remove all particles from the filter. Tap

your finger once to the edge of the large filter paper to remove particles in case they may have been attached to your finger.

m. After about 10 s transfer the large filter paper to the heating plate, the colour development of the Microtracer particles is stopped by the heat.
n. Take the large filter paper off the heating plate with tweezers when it is dry.
o. Label the large filter paper with a pencil.

Note:
Clean the workplace dry after each sample.
The number of Microtracer particles on one filter paper may exceed 200 if the laboratory can prove that more particles per filter can be counted correctly.

3.10. Evaluation

Each Microtracer particle is developed to a colour dot on the large filter paper. The number of colour dots equals the number of particles. The dots are counted by eye or with an appropriate computer aided system (e.g. TraCo image assessment and evaluation system). To yield correct results the statistical evaluation is done in accordance with the Poisson distribution.

1. Evaluation of homogeneity

The following statistical data are relevant:

a. Number of analysed samples (=n)
b. Mean number of Microtracer particles in batch 1 (=X_m)
c. Number of Microtracer particles in different samples, corrected for the mean sample size (=X_n)
d. Number of degrees of freedom of the system (= n – 1)
e. The sum of the squares of the difference between the number of Microtracer particles in different samples (X_n), and the mean number of Microtracer particles in batch 1 (X_m) gives S.
   $S=\sum(X_n - X_m)^2$.
f. Chi squared value (=S/X_m)
g. The probability p in % can be calculated from chi squared and the number of degrees of freedom e.g. with Excel using the CHIVERT function.
   p in % = CHIVERT(chi squared; number of degrees of freedom) × 100
h. Microtracer recovery in % recovery in % = Xm × 100 / number of Microtracer particles added to batch 1

Using the probability p in %, the assessment of the homogeneity is defined as follows:

a.	if p ≥ 25 % it can be conducted that the mixture is excellent. The closer the p value is to 100 % the better the mixture is.
b.	if 5% ≤ p < 25 % it can be conducted that the mixture is good.
c.	if 1 % ≤ p < 5 % no clear statistical conclusion can be made. It is recommended to repeat the test.
d.	if p < 1 % it can be concluded that the mixture is nonhomogeneous.

Example1: Homogeneous mix

Sample number n	Corrected number of particles counted X_n	Difference $X_n - X_m$	Square of difference $(X_n - X_m)^2$
1	100	-13	169
2	100	-13	169
3	124	11	121
4	123	10	100
5	104	-9	81
6	121	8	64
7	119	6	36
8	103	-10	100
9	117	4	16
10	115	2	4
Mean X_m = 113	Sum S = 860		

number of samples: n = 10
number of degrees of freedom: n – 1 = 9
Chi squared: chi squared = 860 / 113 = 7.6
p in %: p in % = CHIVERT(7.6;9). × 100 = 56
Result: The calculated probability (56 %) is higher than 25 %. The mixture is excellent.

Example 2: Non - homogeneous mix

Sample number n	Corrected number of particles counted X_n	Difference $X_n - X_m$	Square of difference $(X_n - X_m)^2$
1	97	-51	2601
2	153	5	25
3	114	-34	1156
4	184	36	1296
5	58	-90	8100
6	155	7	49
7	115	-33	1089
8	181	33	1089
9	255	107	11449
10	164	16	256
Mean X_m = 148	Sum S = 27110		

number of samples: n = 10
number of degrees of freedom: n – 1 = 9
Chi squared: chi squared = 27110 / 148 = 183
p in %: p in % = CHIVERT(183;9) × 100 = 0

Result: The calculated probability (0 %) is below 1 %. The mixture is nonhomogeneous.

Notes on evaluation of data:
First samples of batch 1:
The Microtracer level in the first samples of batch 1 can be lower than in the subsequent samples depending on the sampling place. This effect is called "negative carry-over", because these first samples have a high likelihood of occurrence to be mixed with product from the preceding batch where no Microtracer has been added.

Proceedings for strongly deviating single values:
If the particle count of one sample (X_i) deviates more than 20 % from the mean of all analysed samples (X_m), the analysis of this sample has to be repeated twice. Three different situations may occur:

a. all three analysed particle counts are lying close together (difference less than 20 %), then the first analysis of the three particle counts is chosen for the calculation of the homogeneity.
b. two analysed particle counts are close together (difference less than 20 %), the third analysed particle count varies more than 20 %. The first analysis of the two particle counts which lie close together is chosen for the calculation of the homogeneity.
c. all three analysed particle counts are differing more than 20 % from each other. This means the sample is inhomogeneous. The sample before and after this specific sample has to be analysed. Example: Sample 5 is inhomogeneous, sample 4 and sample 6 have to be analysed. If sample 4 and 6 are fitting to the evaluation of homogeneity, sample 5 is taken out.

1. Evaluation of carry-over

The following statistical data are relevant:

a. Mean sample weight in batch 2 (=w_m)
b. For each sample: number of Microtracer particles for w_m in batch 2
c. The expected number of Microtracer particles for w_m in batch 1 (i.e. 100 % carry-over)
d. For each sample: carry-over level in % (100 x result from b/result from c)
e. Mean carry-over level in % (mean of carry over levels for each sample)

3.11. Reporting

The following will be reported:

a. company specific information (*section 4 of this chapter*)
b. details on sampling (*section 7 of this chapter*)
c. if relevant information on preparation of samples (*section 8 of this chapter*)

For each group of samples:

d. The measured and corrected Microtracer particle counts
e. The relevant statistical data for homogeneity and carry-over, respectively

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