Technical Specifications

TS 1.11 - Control of residues

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GMP+ International

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Welcome

This Feed Certification scheme document helps you to provide feed safety worldwide. By meeting the requirements set by GMP+ International together with our GMP+ Community, we aim to help you get the feed certification you need. Please read the information in this document carefully.

Let's make this work together!

1. Scope of this document

This document specifies:

- the limits for the residues of feed additives and veterinary medicinal products .
- specific requirements regarding the control of residues of the feed additives and veterinary medicinal products
- the methods to measure the carry-over and homogeneity

The requirements in this document are **in addition** to those set out in the R 1.0 *Feed Safety Management Systems Requirements*.

2. Limits for critical residues

2.1. General

1. Any company, participating in the GMP+ FSA module, whether located inside or outside Europe, must control the use of feed additives and veterinary medicinal products. The GMP+ certified company must ensure that (residues of) these feed additives and veterinary medicinal products are not present in other than the intended feed or do at least not exceed maximum limits (the so-called residue limits) in other than the intended feed.

2. The GMP+ certified company must ensure compliance with the residue limits stated in this document. The residue limits in this document are mainly based on European Union legislation. These residue limits are adopted into the GMP+ FSA module. In principle, the residue limit of a certain feed additive or veterinary medicinal product is a percentage of the maximum content, which is allowed to mix into feed. In the European Union Feed legislation residue limits are laid down, based on the factors in the next table.

Feed additive/Veterinary medicinal product	Max. percentage (%)	Remark
Coccidiostats	1	For critical feed
	3	For other feed
Antibiotics	2.5	

These limits have been specified in the tables in this chapter. Also, for a number of other substances residue limits have been specified in this tables, mainly calculated with a 'max 2.5%-factor'.

3. In other parts of the world, also other substances (non-EU registered coccidiostats, antibiotics and other products as growth promoters, veterinary medicinal products) are allowed to use in feed. These products must be classified as 'Other substances, for which a withdrawal time has been established' in the table below.

Helpful tip:

'Other substances for which a withdrawal time has been established' are products
- which are deliberately added to the feed with the intention to influence performance, production or health of the animal, and
- which can be found in the animal products (meat, milk or egg), and can be harmful when consumed by man, and
- for which subsequently a withdrawal time has been defined.

2.2. Limits for residues of feed additives

Feed additives	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
Lasalocid A sodium	Feed materials	1,25
	Compound feed for: • dogs, calves, rabbits, equine species, dairy animals, laying birds, turkeys (> 16 weeks) and chickens reared for laying (> 16 weeks) • chickens for fattening, chickens reared for laying (< 16 weeks) and turkeys (<16 weeks) for the period before slaughter in which the use of Lasalocid A sodium is prohibited (feed during withdrawal time) • pheasants, guinea fowl, quails and partridges (except laying birds) for the period before slaughter in which the use of Lasalocid A sodium is prohibited (feed during withdrawal time), • other animal species	1,25 1,25 1,25 3,75
	Premixtures for use in feed in which the use of Lasalocid A sodium is not authorised	([1])
Narasin	Feed materials	0,7
	Compound feed for: • turkeys, rabbits, equine species, laying birds and chickens reared for laying (> 16 weeks) • other animal species	0,7 2,1
	Premixtures for use in feed in which the use of Narasin is not authorised.	([1])
	Feed materials	0,7

Feed additives	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
Salinomycin sodium	Compound feed for: • equine species, turkeys, laying birds and chickens reared for laying (> 12 weeks) • chickens for fattening, chickens reared for laying (< 12 weeks) and rabbits for fattening for the period before slaughter in which the use of Salinomycin sodium is prohibited (feed during withdrawal time) • other animal species	0,7 0,7 2,1
	Premixtures for use in feed in which the use of Salinomycin sodium is not authorised.	([1])
Monensin sodium	Feed materials	1,25
	Compound feed for: • equine species, dogs, small ruminants (sheep and goat), ducks, bovine, dairy animals, laying birds, chickens reared for laying (> 16 weeks) and turkeys (> 16 weeks) • chickens for fattening, chickens reared for laying (< 16 weeks) and turkeys (< 16 weeks) for the period before slaughter in which the use of Monensin sodium is prohibited (feed during withdrawal time) • other animal species	1,25 1,25 3,75
	Premixtures for use in feed in which the use of Monensin sodium is not authorised.	([1])
Semduramicin sodium	Feed materials	0,25
	Compound feed for: • laying birds and chickens reared for laying (> 16 weeks) • chickens for fattening for the period before slaughter in which the use of Semduramicin sodium is prohibited (feed during withdrawal time) • other animal species	0,25 0,25 0,75
	Premixtures for use in feed in which the use of Semduramicin sodium is not authorised.	([1])
Maduramicin ammonium alpha	Feed materials	0,05
	Compound feed for: • equine species, rabbits, turkeys (> 16 weeks), laying birds and chickens reared for laying (> 16 weeks) • chickens for fattening and turkeys (< 16 weeks) for the period before slaughter in which the use of Maduramicin ammonium alpha is prohibited (feed during withdrawal time) • other animal species	0,05 0,05 0,15
	Premixtures for use in feed in which the use of Maduramicin ammonium alpha is not authorised.	([1])
Robenidine hydro-chloride	Feed materials	0,7
	Compound feed for: • laying birds and chickens reared for laying (> 16 weeks)	0,7 0,7

Feed additives	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
	• chickens for fattening, rabbits for fattening and breeding and turkeys for the period before slaughter in which the use of Robenidine hydrochloride is prohibited (feed during withdrawal time) • other animal species	2,1
	Premixtures for use in feed in which the use of Robenidine hydrochloride is not authorised.	([1])
Decoquinate	Feed materials	0,4
	Compound feed for: • laying birds and chickens reared for laying (> 16 weeks) • other animal species	0,4 1,2
	Premixtures for use in feed in which the use of Decoquinate is not authorised	([1])
Halofuginone hydro-bromide	Feed materials	0,03
	Compound feed for: • laying birds, chickens reared for laying and turkeys (> 12 weeks) • chickens for fattening and turkeys (< 12 weeks) for the period before slaughter in which the use of Halofuginone hydro bromide is prohibited (feed during withdrawal time) • other animal species	0,03 0,03 0,09
	Premixtures for use in feed in which the use of Halofuginone hydro bromide is not authorised.	([1])
Nicarbazin	Feed materials	1,25
	Compound feed for: • equine species, laying birds and chickens reared for laying (> 16 weeks) • other animal species	1,25 3,75
	Premixtures for use in feed in which the use of Nicarbazin (in combination with Narasin) is not authorised.	([1])
Diclazuril	Feed materials	0,01
	Compound feed for: • laying birds, chickens reared for laying (> 16 weeks) • rabbits for fattening and breeding for the period before slaughter in which the use of Diclazuril is prohibited (feed during withdrawal time). • other animal species other than chickens reared for laying (< 16 weeks), chickens for fattening, guinea fowl and turkeys for fattening.	0,01 0,01 0,03
	Premixtures for use in feed in which the use of Diclazuril is not authorised.	([1])
	Note: • Chickens for fattening: feed given to these chickens from 5 days before slaughter • Turkeys for fattening: feed given to these turkeys from 5 days before slaughter • Pigs: feed given to pigs from 28 days before slaughter	

Feed additives	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
For other coccidiostats	For all feed	1% of the max. content, which is approved to mix in a feed.

(1) The maximum level of the feed additive/veterinary medicinal product in the premixture must not result in a level of that feed additive/veterinary medicinal product higher than 50 % of the maximum levels established in the feed when the instructions for use of the premixture are followed.

2.3. Limits for residues of veterinary medicinal products

Veterinary medicinal products	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
Sulfadiazine sodium	Compound feed for: • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs • Dairy animals	 5 8 1 1
Sulfamethoxazol	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs • Dairy animals	 5 8 1 1
Doxycycline	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs Dairy animals	 8 8 10 1 batch[2]
Oxytetracycline	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs • Dairy animals	 1 10 10 1 batch[2]
Ivermectine	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs • Dairy animals	 0,1 0,1 0,1 1 batch[2]
Tiamuline	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening	 1 8

Veterinary medicinal products	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
	• Pigs • Dairy animals	10 1 batch[2]
Tilmicosine	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs • Dairy animals	 1 4 10 1 batch[2]
Trimethoprim	Compound feed for • Laying birds • Chickens for fattening and Turkeys for fattening • Pigs • Dairy animals	Linked to Sulfadiazine and therefore sufficiently ensured

(2) Dairy cow feed must not immediately be produced on a production line which has just produced feed with these feed additives/veterinary medicinal products.

2.4. Limits for residues of other Feed additives / Veterinary medicinal products

Veterinary medicinal products	Feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12 %
Other substances, for which a withdrawal time has been established[3]	All other feed for animals that produce animal products like • Laying hens • Milk producing cows, goat, sheep, etc. • Chickens for fattening and turkeys for fattening (feed given to these chickens for fattening and turkeys for fattening from 5 days before slaughter) • Pigs (feed given to these pigs from 28 days before slaughter)	1

(3) Examples: Flubendazol, Carbadox, Olaquindox.

3. Additional requirements for the control of residues

3.1. General / Installation

A GMP+ certified company can apply several control measures to ensure that residues of critical feed additives and veterinary medicinal products are not exceeding the limits laid down in the table in § 2.2, § 2.3 and § 2.4.

Helpful tip 1:

Possible control measures can be:

- Not using any feed additive or with a residue limit at all.
- Separation between locations where feed additives/ veterinary medicinal products are used, and where they are not used.
- Separation between production equipment and internal transport facilities (with and without critical feed additives/veterinary medicinal products) within a location.
- Choose less critical feed additives or veterinary medicinal products
- Transport of the first 50-100 kg of produced feed (after medicated feed) to be disposed as waste or to be used as rework in feed with the same feed additive or veterinary medicinal product(s).
- Using specific equipment (internal transport, mixer, filter).
- Proper maintenance and cleaning of the equipment.
- Dosing veterinary medicinal products in the mixer or bulk blending equipment.
- Use of a fixed dosage sequence for micro components.
- Use of short ways for transport / use proper idle times.
- Avoiding of places where products can stay behind.
- Applications of a strict production sequence/flushing. See for this § 3.2.

In all the GMP+ standards is laid down that control measures must be validated and their effectiveness must be verified with an appropriate frequency ('HACCP principles' must be applied). This includes the control measures for controlling residues of veterinary medicinal products / additives.

When using a specific production sequence for controlling the residue limits, specific validation & verification is required. See for this § 3.2.4.

Helpful tip 2:

Validation: can be applied in accordance with the common HACCP principles. The GMP+ certified company should be sure that application of a certain control measure gives the expected result (= no residues or at least below the limits). Results of analytical research is very helpful here. After each essential change, control measures should be reconsidered and – if necessary - updated and validated.

3.2. Control of residues via production sequence

3.2.1. General

A very common method used for controlling residue levels is to flush the production installation after a veterinary medicinal product or a feed additive is used, thus 'cleaning' an installation.
When using this method, a strict production sequence must be calculated and applied, with enough flushing batches to ensure that residue levels are not exceeded.

If feed is used for flushing after a medicated feed or feed with a coccidiostat is produced, it must be ensured that the residue level of the veterinary medicinal products or the feed additives in this feed does not exceed the limits.

If a feed material is used for flushing, it must be used or processed with great care afterwards. A risk analysis must proof the correct use of this feed material. This feed material might be used in a feed with the same coccidiostat or antibiotic. It can also be disposed of as waste.

The calculation, which is based on the degree of carry-over of a production installation, must result in expected levels (calculated) of residues of critical feed additives and veterinary medicinal products in successive batches, after the batch in which a company has used a critical feed additive or veterinary medicinal product.

Note: The maximum level of the feed additive/veterinary medicinal product in the premixture is the concentration which must not result in a level of this feed additive/ higher than 50 % of the maximum levels established in the feed when the instructions for use of the premixture are followed.

Helpful tip:

For example: the maximum residue limit of a feed additive for feed is 1 ppm. The premixture may contribute max. 0,5 ppm in the feed (50%). When the premixture must be mixed in the feed with 5%, according to the instructions, the maximum residue limit for the premixture is 10 ppm.

Also feed additives like copper and zinc have maximum limits which must not be exceeded. See for this TS 1.5 *Specific feed safety limits*.

3.2.2. Carry-over percentage of installation

General
A testing procedure laid down in chapter 4 of this document must be used for measuring the carry-over percentage of an installation. All production, processing and transport lines in a facility, which can contribute to the carry-over, must be tested. See for more details chapter 4.

Frequency
The minimum frequency of measuring the carry-over in production and transport lines depends on the (feed and premixtures with) feed additives and veterinary medicinal products which the GMP+ certified company processes and whether he processes feed for which a residue limit has been established.

If the GMP+ certified company processes or transports products (or feed containing these products) for which a specific residue limit has been laid down in the table in § 2.2, 2.3 and § 2.4, the percentage of carry-over must be known for the lines on which these products are processed, produces or transported. If the GMP+ certified company has such production lines, he must measure carry-over at least once per two years.

When processing or transporting any other product, which may give residues in animal products, the GMP+ certified company must measure the carry-over at least once.

The carry-over must be re-measured in the event of major changes to the installation.

3.2.3. Safety factor

The actual processing properties of a critical feed additive or veterinary medicinal product may be different from the tracers that are used during the measuring of the carry-over percentage with one of the methods that are laid down in chapter 4.

To achieve more guarantees that the real residue levels do not exceed the calculated (expected) residue levels, a GMP+ certified company may use in the calculation of the production sequence a so-called safety factor. When using the safety factor in the calculation, a GMP+ certified company may lower the verification frequency. See for this § 3.2.4.

The default safety factor to be used is “3” . However, in the tables in § 3.2.5 for a number of critical feed additives and veterinary medicinal products, other safety factors are laid down.

Helpful tip:

These safety factors are determined based on a so-called relative wall adhesion factor, measured with a test specially developed for this purpose. If a GMP+ certified company wants to use this test for establishing the specific wall adhesion factor, GMP+ International can be contacted.

3.2.4. Validation and Periodically verification (monitoring)

Validation
Any calculated production sequence must be properly validated to show effectiveness in controlling residue levels. At least two samples (of feed with products with residue limits in the table in § 2.2, § 2.3 and § 2.4) must be taken and analysed.

When the degree of carry-over has been re-measured and the production sequence has been recalculated, a new validation must be carried out.

Verification
To prove ongoing effectiveness of the used production sequence, the GMP+ certified company must monitor by means of analysing the residue levels in relevant feed:

a) When not using the safety factor in the calculation of the production sequence: four samples per year must be analysed.
b) When using the safety factor in the calculation of the production sequence: two samples per year must be analysed.

Helpful tip:
The GMP+ certified company has a choice here. If the safety factor is used, the minimum monitoring frequency per year is lower.

Verification must be carried out by means of analysing the residue levels of the specific veterinary medicinal product or feed additive. When more veterinary medicinal products or feed additives are used in the production, the one with the highest safety factor must be analysed as part of the verification.

Analysis must be carried out by a laboratory that is approved as such (See for this TS 1.2 *Purchase*). The detection limit of the method used must be appropriate to decide if the established system of production sequence is sufficient.

3.2.5. Additional information about the safety factor

Name of Coccidiostats and histomonostats, which have been tested with the so-called wall-adhesion test	Producer	Safety factor	
		Pigs	Other
Compound of Narasin and Nicarbazin			
Maxiban G 160 premix	Elanco GmbH	3	1
Lasalocid sodium			
Avatec 15% CC	Zoetis Belgium S.A.	1	1
Robenidine-hydrochloride			
Cycostat 66G	Zoetis Belgium S.A.	1	1
Monensin-sodium			
Elancoban G200 premix	Elanco GmbH	1	1
Coxidin (5 1 701)	Huvepharma NV Belgium	1	1
Narasin			
Monteban G100 premix	Elanco GmbH	1	1
Halofuginone-hydro bromine (764)			
Stenorol	Huvepharma NV Belgium	1	1
Diclazuril			
Clinacox 0,5 % Premix	Elanco GmbH	2	2
Salinomycin-sodium			
Sacox 120 microGranulate	Huvepharma NV Belgium	1	1

4. Methods for measuring carry-over

4.1. Introduction

To measure the carry-over, the GMP+ certified company must use the protocols, which are laid down in this part of the document.

The report on the carry-over measurement must comply with further conditions. See 'Report' (page 17).

Note: It is permissible for GMP+ certified companies to deviate from the method laid down as long as the principles as stated in § 4.2 are not affected and it can be demonstrated that equivalent results will be obtained.

In some countries, in legislation special requirements to measure the carry-over are laid down. The results of these measurements are accepted to demonstrate compliance with the GMP+ requirements.

4.2. General basic principles with respect to the measurement of carry-over

When measuring the carry-over of additives in an installation there must be a prior examination using a flowchart (graphic reproduction of e.g. the factory) and the actual situation in the factory of which parts of the factory may be relevant for carry-over.

A basic principle in determining carry-over in a company is that the degree of carry-over as a result of return flows is known and is controlled.

Carry-over points

Carry-over in a (compound feed) factory may occur in the following processes.

1. **The filling of premix silos**
   The filling of the premix silos may be the cause of carry-over. The flowchart can be used to find out whether there are reasons to suppose that carry-over occurs here. Critical points are common transport systems, chutes, separation systems and filters.

   In mechanical transports such as mass transports, elevators and screw conveyors, carry-over always occurs and it is sensible to measure this carry-over. Also, sufficiently long idle times (10 minutes) must be taken into account.

For the pneumatic filling method with separate filters for each silo, no account needs to be taken of carry-over. If there is a common filter then the filter must, for at least 10 minutes after unloading, be knocked on the same silo as that in which the filling took place.

There must be an instruction for the dumping sequence so that undesired mixing does not take place.

In this situation it must be certain that unacceptable residue levels no longer occur.

2. Dosage, grinding and mixing line

The greatest amount of carry-over of additives and veterinary medicinal products occurs in the dosage process (addition of additives or veterinary medicinal products) / (possibly grinding) / mixing / transport and storage of the product in meal form in a finished product cell or a pressed meal cell.

The place where premixes are added must be as close to the mixer as possible. It is important that the measured substance is added at the same place as where the additive and veterinary medicinal products were added.

3. Press line

A considerable amount of carry-over can occur in the press line. The carry-over increases as the press die is bigger. In addition, interim bunkers containing stocks can be a source of carry-over.

An item for attention is the return flows which are brought back directly into the pressed meal silo during pelletising.

4. Loading and transport

During storage, loading and transport of a finished product there will only be carry-over of any importance for highly critical additives and veterinary medicinal products (for example nicarbazine and sulfa-veterinary medicinal products). In these cases a mandatory working sequence must be used.

An item for attention is the processing of the sievings from the bulk load. Possible processing of such sievings must at least comply with the feed legislation and must therefore be processed in a careful and controlled fashion. Any sievings of medicated feed may not be reprocessed.

If the undesired carry-over of critical additives and veterinary medicinal products may be expected then the GMP+ certified company may take the following measures:

a) the drawing up of a mandatory production (working) sequence
b) additional measures in the event of product changes

c) the production of feed with critical additives and veterinary medicinal products on another line
d) switching to less critical agents.

Measurement points for carry-over

The major causes of carry-over are the dosage / grinding / mixing line and the press line. The carry-over must be known if both feed with critical additives and veterinary medicinal products as feed with a maximum carry-over level are produced on these lines. In order to establish this reliably the following measurement points are important:

After the mixer, but as close as possible to the mixer for the measurement of the output content of the mixer:

a. at the entry to the pressed meal cell in kernel production or the finished product cell in meal production, for the measurement of the carry-over on the dosage / grinding / mixing line
b. at the entry to the finished product cell in kernel production for the measurement of carry-over on the press line.

Carry-over which is determined in this way is considered to be the installation carry-over.

Report

Good reporting on the measurement is important to be able to apply the results unambiguously when determining measures and during supervision of the correct implementation. This must be based on a well thought out and properly described protocol which has been talked through in advance with those who will implement it and on a careful implementation of this protocol. At least the following items must therefore be laid down:

i. date
ii. who is responsible for the carry-over measurement
iii. description of the method used
iv. a plan of the installation with an indication of
    a) grinding, mixing and press lines which were measured
    b) the place where the measured substance was added
    c) sampling points
v. the number and size of the samples
vi. the sampling time interval
vii. analysis results
viii. proper calculation of the carry-over
ix. any sample pre-handling such as grinding, homogenisation, splitting and/or putting together

New measurement substances

New measurement substances must be admitted on the basis of an assessment where there has been validation with respect to the reference method The validation report must contain at least the following elements:

i. name and address details of the submitter and measurement agency;
ii. motivation/problem description;
iii. characteristics with respect to the
    a) Feed installation to be used (including mixer/press installation/cooler);
    b) The reference measurement substances and the measurement substances to be examined;
    c) Sampling plan for the samples to be taken in the various flush batches;
    d) Sample preparation in the laboratory;
    e) Analysis methods to be used;
    f) Statistical methods to be used.
iv. analysis results;
v. statistical processing of the analysis results;
vi. conclusions;
vii. references.

The report may be submitted for assessment by an expert panel to GMP+ International.

4.3. 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

1. APPLICATION AREA

The testing procedure was developed for the determination of the carry-over which occurs in compound feed production companies. The carry-over of the dosing lines of raw materials and the carry-over of the components which are added via the premixtures are determined separately.
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 uniform mixes can be produced using the installation (see item 9).

2. DEFINITIONS

Carry-over
Carry-over means that part of the previous batch of feed remains in the production and transport system and gets into the following batches.

Carry-over level
The carry-over level is defined as the amount of a component from a previous batch, expressed as a percentage, which gets into the following batch of feed (of the same size). The carry-over level can be measured for a section of the installation (for example the pressed meal bunkers) or for the whole installation.

3. 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.

4. 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).

5. 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.

6. IMPLEMENTATION OF THE TESTING PROCEDURE

6.1.a. 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.

6.1.b. 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.

6.2.a. 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 (sampling).
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 (sampling) and then pelletised (sampling).

6.2.b. 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 b) and c). 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:

4. during the first 30 seconds as many sub-samples as possible are collected in a bucket; a mix sample is made from these
5. for the second 30 seconds: idem
6. 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:

7. during the first minute as many sub-samples as possible are collected in a bucket; a mix sample is made from these
8. during the second minute: idem
9. 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.

6.3 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.

7. 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.

8. 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)

$$\text{for CP} = \frac{95.6 - 80.3}{420 - 80.3} \times 100 = \frac{1.530}{339.7} = 4.5\%$$

$$\text{and for Mn} \frac{64.7 - 4.4}{2{,}006 - 4.4} \times 100 = \frac{6.030}{2{,}001.6} = 3\%$$

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.

9. THE MEASUREMENT OF UNIFORMITY

In order to determine the extent to which the installation produces uniform 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 uniformity.

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 uniformity 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 uniformity 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.

10. 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	5 mg/kg	2 - 8 mg/kg	0,16 - 0,18%
	1	23	20 - 26	0,8 - 1,2
	3	59	56 - 62	2,7 - 3,4
	5	95	90 - 100	4,5 - 5,6
	10	185	176 - 194	9 - 11,1
	15	275	261 - 289	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	80 g/kg	79.2 - 80.8 g/kg	- 0,25 - 0,25
	1	83,2	82,4 - 84,0	0,7 - 1,3
	3	89,6	88,7 - 90,5	2,6 - 3,4
	5	96	95,0 - 97,0	4,5 - 5,5
	10	112	110,9 - 113,1	9,4 - 10,6
	15	128	126,7 - 129,3	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).				

4.4. Testing procedure for the measurement of carry-over in Premixtures 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 chapter 4.2

2. CARRY-OVER PROCESS

a. The carry-over process to be measured relates to the point where the additives and/or animal veterinary medicinal products are added to the bulk vehicle load or the bag filling.
b. Measurement of the carry-over must be carried out for each production line in the installation.
c. The measurement must 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:
the microtracers FSS-Lake and F-Lake

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:

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

4.5. Testing procedure for the process accuracy of compound feed with microtracers

1. FIELD OF APPLICATION

This procedure may be used in the feed production industry for determining the homogeneity in premixtures 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.

2. DEFINITIONS

Microtracer particles:	Very fine elementary iron particles 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 600.000 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. 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.

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

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:

5.1 HOMOGENEITY (BATCH 1)

Dosing of the Microtracer particles:

Information required:

a) accuracy to be checked (e.g. 1:100 000)
b) size of Microtracer premix $[g]$
c) batch size of the test mix$[g]$
d) number of Microtracer particles per gram (from certificate of analysis)

Calculations:

e) Weight of pure Microtracer to be added:
batch size × accuracy = weight of Microtracer $[g]$ 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.)

f) The total number of added Microtracer particles is calculated:
weight of Microtracer $[g]$ × number of Microtracer particles per gram = number of Microtracer particles added
g) 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 600 000 particles per gram

Calculations:

h) 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).
i) Total number of particles: 10 × 600 000 = 6.000.000 particles. The complete Microtracer premix is added to the first 1000 kg test batch in the feed production.
j) theoretical concentration in first batch: 6 000 000 / 1 000 000 g = 6 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 × 6 particles per g =120 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.

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 about half of the sample weight is analysed for samples C 1 – C 3 (see section 9).

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.

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

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

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.

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.

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 (110°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.

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. <u>Evaluation of homogeneity</u>

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 20g 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 % = X_m × 100 / number of Microtracer particles added to batch 1

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

i) 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.
j) if 5% ≤ p < 25 % it can be conducted that the mixture is good.
k) if 1 % ≤ p < 5 % no clear statistical conclusion can be made. It is recommended to repeat the test.
l) if p < 1 % it can be concluded that the mixture is nonhomogeneous.

The Microtracer recovery must be 100 % ± 15 %. Reasons for a low recovery rate are usually found in the production installation, i.e. if not all of the Microtracer premix did reach the mixer or strong external magnets take out a minor portion of the Microtracer (this does not influence the test result).

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 uniformity.
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 uniformity.
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.

2. Evaluation of carry-over

The following statistical data are relevant:

a) Mean sample weight in batch 2 (=w_m)
b) For each sample: mean 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 %
e) Mean carry-over level in %

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

12 LITERATURE

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5. Methods for measuring homogeneity of dry mixtures [1]

5.1 Introduction

The GMP+ certified company mixes feed materials, feed additives and veterinary medicinal products uniformly through the feed in accordance with the requirements in TS 1.1 *Prerequisite programme* and TS 1.10 *Operational activities*. Measurement of the homogeneity of mixtures is in accordance with the protocols, which are laid down in this chapter.

5.2 Frequency

A homogeneity test must be performed on each mixing installation. This test must done at least,

a) At first use of the installation.
b) At every significant change to the installation.
c) Every four years.

5.3 Measurement of homogeneity

5.3.1 General

The measurement of homogeneity is statistically determined, by making use of direct or indirect methods.

5.3.2 Direct methods

Direct methods for measuring homogeneity are based on the **counting of particles**. So called microtracers are used as a measuring substance. Two different microtracers are suitable for the homogeneity analysis: Microtracer F and Microtracer FSS. Application of these methods lead to analysis results, which are analyzed as Poisson distributions. Homogeneity is expressed in terms of probability (p). The application of these methods must be in accordance with the description of the method in § 4.5 above.

[1] *Dry compound feed or dry premixtures. Mixtures of liquid feed, emulsions, suspensions are out of scope.*

Helpful tip:

Example of the calculation of homogeneity with the direct Microtracer FSS method.
Dosage of Microtracer FSS is 10 g per ton of test mix.

Homogeneity Batch	100 % filling dry, 50 Hz	
Planned batch size:	5392	
Overweight:	120	
Real batch size:	5512	
Addition of Microtracer Premix:	directly through an opening in the mixer on top of the mix	
Time for emptying of pre-bin to mixer:	15 s	
Dry Mixing Time:	90 s	
Addition of Liquids:	0 s	
Wet mixing time:	0 s	
Total mixing time:	105 s	
Sampling place:	after reddler before elevator	
Number of Samples:	22	
Sample Assayed, g:	20	
Tracer Color:	FSS-red lake	
Tracer Used per Metric Ton, g:	9,78	

Analytical results:

Sample No.	Sampling time [s]	Microtracer Particle Count	Sample Assayed [g]	Corrected Particle Count
1	0	74	19,46	76
2	10	102	21,50	95
3	20	92	21,29	86
4	30	97	21,59	90
5	40	97	21,27	91
6	50	92	20,54	90
7	60	103	21,01	98
8	70	92	20,69	89
9	80	100	21,06	95
10	90	87	21,01	83
11	100	77	20,94	74
12	110	85	21,11	81
13	120	95	20,01	95
14	130	83	19,97	83
15	140	83	21,97	76
16	150	88	20,30	87
17	160	85	20,68	82
18	170	82	20,67	79
19	180	83	20,02	83
20	190	73	19,97	73
22	210	82	20,09	82
24	230	82	20,05	82

Statistical Evaluation:

Number of Data	22
Degrees of Freedom	21
Mean, Particles	85
Standard Deviation, +/- Particles	7
c^2 Chi-square =	13,02
Probability, %	**91**
Tracer Recovery, % =	104

Mixing uniformity:
Mixing is excellent.

Table 1: Definitions for Mixing Uniformity

	direct tracer	indirect tracer
	Microtracer	example: Manganese
	Probability p	Coefficient of Variation cv
Mixing is excellent	> 25 %	< 5 %
Mixing is good	> 5 % - < 25 %	> 5 % - < 8 %
Mixing is acceptable	> 1 % - < 5 %	> 8 % - < 12 %
Mixing is incomplete	< 1 %	> 12 %

5.3.3 Indirect methods

Indirect methods for measuring homogeneity are based on the **determination of a concentration of a substance** (Microtracer RF Lake Blue or additive). Indirect methods are:

a) Method with tracer Microtracer RF Lake Blue
b) Method with tracer composed of an additive (Salinomycin)
c) Method with a mix of manganese oxide and a protein-rich and a protein-poor mix

Application of these methods lead to analysis results, which are considered as being normal distributions. Homogeneity is given by the coefficient of variation (CV). The application of the above indirect methods must be in accordance with the descriptions in § 4.5.

5.4 Interpretation of homogeneity results

Depending on the method used, the results must be interpreted based on the limits in the next tables.

Determination of homogeneity by means of direct methods

Probability p	**Assessment**
p ≤ 1%	Insufficient
1% < p < 5%	Probably significant deviation. No unambiguous statement can be made. The test must be repeated.
P ≥ 5%	Good homogeneity

Determination of homogeneity by means of indirect methods

Coefficient of variation CV	**Assessment**
CV ≤ 8%	Good homogeneity
8% < CV < 12%	Acceptable homogeneity
CV ≥ 12%	Insufficient

In case the homogeneity of the mixture is assessed as insufficient, the GMP+ certified company must:

a) Report on the probable cause(s)
b) Carry out corrective measures
c) Perform a new homogeneity test in order to verify that the measures taken lead to a good homogeneity.

Appendix: Additional information about the safety factor for a number of veterinary medicinal products, which are approved for the Dutch market

Name	Producer/importer	Safety factor	
		Pigs	Other
Doxycyclinehyclaat/broomhexinehydrochloride			
Feedmix Doxy-B	Dopharma Research B.V.	2,5	2,5
Pulmodox 5% Premix	Virbac Laboratories	2,5	2,5
Doxyprex	Industrial Veterinaria S. A.	2,5	2,5
Sulfadiazinenatrium/Trimethoprim			
Feedmix Trim/sul 80/420	Aesculaap BV	3	3
Trimethosulf premix	Eurovet Animal Health B.V.	3	2
Feedmix sulfatrim	Dopharma Research B.V.	3	3
Sulfamethoxazol/Trimethoprim			
Feedmix TS	Dopharma Research B.V.	3	3
Vetmulin 10% premix for medicated feeds	Huvepharma N.V.	1	1
Tilmicosinefosfaat			
Tilmovet 10%, premix for medicated feed for pigs	Huvepharma N.V.	1	1
Tilmovet 4%	Huvepharma N.V.	1	1
Tilmovet 20%, premix for medicated feeds for pigs	Huvepharma N.V.	1	1
Tylosinefosfaat			
Pharmasin 20 mg/g premix	Huvepharma N.V.	1	1
Pharmasin 100mg/g premix for medicated feeds for pigs, chickens for fattening and chickens reared for laying	Huvepharma N.V.	1	1
Pharmasin 250mg/g premix for medicated feeds pigs, chickens for fattening and chickens reared for laying	Huvepharma N.V.	1	1
Flubendazol (different mixes)		3	3
Ivermectine (different mixers)		3	3

Feed Support Products

That was a lot of information to digest and one might ask, what is the next step? Luckily we can offer support for the GMP+ Community when doing this. We provide support by means of various tools and guidances but as each company has a shared responsibility to feed safety, and therefor tailor-made solutions cannot be offered. However, we do help by explaining requirements and provide background information about the requirements.

We have developed various supporting materials for the GMP+ Community. These include various tools, ranging from Frequently Asked Questions (FAQ) lists to webinars and events.

Supporting materials related to this document (Guidelines and FAQ's)
We have made documents available which give guidance to the GMP+ requirements as laid down in the module GMP+ FSA and GMP+ FRA. These documents give examples, answers to frequently asked questions or background information.

Find our Feed Support Products here:

Guidelines
More information: https://gmpplus.org/en/feed-certification-scheme-2020/gmp-fsa-fra-certification/support/

At GMP+ International, we believe everybody, no matter who they are or where they live, should have access to safe food.

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